SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Highly Commended

Scientific Inquiry

Year 11-12

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The Effect of *Cinnamomum Verum* Oil Concentration on the Germination of *Triticum Aestivum* Seeds

Aim

To determine the most effective concentration of cinnamon (*Cinnamomum verum)* oil (%) at inhibiting the germination and rate of growth (%) of ryegrass (*Lolium rigidum)* seeds, investigated through the use of wheat (*Triticum aestivum)* seeds.

Personal Engagement

Where I grew up in the Barossa Valley, I often saw hectares of land being sprayed with herbicides to target weed growth. However, I noticed that a large volume of the herbicide would even hit the surrounding crops. This led me to wonder about the potential harm these herbicides may be having on the surrounding crops, environment and local wildlife. As a result, I researched environmentally friendly herbicides that could be used to inhibit weed growth and discovered the potential of essential oils.

Introduction

With increased interest in crop productivity, many farms are applying synthetic fertilisers to kill weed species that may destroy their crops (Dolores and Amparo, 2018). Across Australia, weeds have been shown to impose $5 billion damage to the agricultural economy. This cost includes the mechanical and labour measures implemented to reduce weed growth, and loss of income from reduced product quality and space available for seed growth. Out of all weeds, the costliest seeds have been *L. rigidum* seeds (Mcleod, 2018).

L. rigidum, endemic to the temperate regions of Europe and Asia, is a tufted grass that is highly competitive and able to produce an extremely high number of seeds per plant (Kloot, 1983). *L. rigidum's* hairless and bright green narrow leaves begin to grow from late Autumn to early Spring. When fully grown it can produce up to 45,000 seeds per square metre. Further, it hosts the bacteria *Clavibacter spp.* causing it to be poisonous to any livestock that eats it (Peltzer, 2020). *L. rigidum* is mostly wind-pollinated but can also self-pollinate. These features contribute to its fast-growing nature and classification as an invasive weed species in Australia (Chastain, 2013).

Currently, glyphosate is the most common synthetic herbicide used in cropping systems to kill weeds. However, due to its overuse, glyphosate-resistant crops are developing (Yanniccari, 2017). These synthetic herbicides are a potential hazard to human health as they may cause skin irritancy after short-term exposure or nerve axon damage after long-term exposure (Dolores and Amparo, 2018). Glyphosate can also be damaging to the environment, as it can contaminate the groundwater and surface water or affect wildlife indirectly by altering the vegetation (U.S. Fish & Wildlife Service, 2009).

Consequently, scientists are researching herbicides with limited negative impacts on the environment but which can still control weed growth. One such herbicide which is being explored is *C. verum* essential oil - cinnamon. This is because essential oils may contain natural chemicals called allelochemicals. Allelopathy involves the study of how secondary metabolites (allelochemical compounds) affect the biological system. These allelochemical compounds, produced by plants, algae, bacteria and fungi, can directly or indirectly release phytotoxic effects to limit competing plant's growth in the surrounding environment. Therefore, along with essential oils ability to rapidly decompose in the environment, scientists view them as a potential herbicide to be used in sustainable agriculture (Cavalieri and Caporali, 2010). *C. verum* oil, usually derived from the bark of the *Cinnamomum verum* tree and the *Cinnamomum cassia* tree, has been shown to contain allelochemicals (Wilson, 2019). As *C. verum oil* has also been able to control a broad spectrum of weeds, its vast application makes it more likely to be used in the future, and therefore this essential oil was chosen to be explored in this experiment (Choi and Hwang, 2013).

To investigate the effect of *C. verum* oil on seed germination, 10 petri dishes containing cotton wool and 10 wheat seeds will be sprayed with varying concentrations of *C. verum* oil (Campiglia *et al.*, 2007). As *L. rigidum* is a weed species, the more ethical choice of *T. aestivum* seeds will be used in their place because in the early stages of growth they share similar characteristics such as its green colour, hairless leaves and fibrous root system (Acevedo *et al.*, 2002). An experiment conducted by Tworkoski (2002) investigated the effect of *C. verum* oil at concentrations 0.0%, 0.5%, 1.0% and 2.0% (v/v). However, as that experiment did not explore germination between concentrations 0.0% and 0.5%, the following concentrations, 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500%, were chosen for this experiment and 2 petri dishes for each concentration will be sprayed. Over 6 days, the number of seeds that have germinated will be counted. Once the seeds have initially germinated, the petri dishes will be placed on the windowsill because sunlight is a key factor in supporting growth beyond germination. The plumule was chosen rather

than the radicle due to the difficulty of measuring the root full length within the shallow petri dish in which it was grown. The plumule also develops into the future shoot, including the stems and leaves, which indicates whether *C. verum* oil could be an effective herbicide (Panawala, 2017). Overall, the statistical test that is the Pearson's r correlation test, will be used to determine if there is a statistical difference between the following pairings: the number of germinated *T. aestivum* seeds and the concentration of *C. verum* oil; and the rate of growth of the plumule and concentration of *C. verum* oil.

Research Question

How does the concentration of *Cinnamomum verum* oil (0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500%) affect the germination and growth of *Lolium rigidum* seeds (%)?

Hypothesis

It is hypothesised that *C. verum* oil at a concentration of 0.2500% will be most effective at inhibiting the rate of seed germination and plumule growth of *L. rigidum* seeds. This is hypothesised because allelochemical compounds, contained within *C. verum* oil, can release phytotoxic effects which limit weed growth (Cavalieri and Caporali 2010).

Variables

Independent: The independent variable is the concentration of *C. verum* oil. The varying concentrations are as follows: 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500%. These 8 concentrations are made by mixing different volumes of *C. verum* oil and distilled water.
Dependent: The dependent variable is the number of seeds that have germinated in 6 days and of the germinated seeds, the length of each seed's plumule (mm). The number of seeds will be counted and a calliper will be used to measure the plumule length.

Controlled:

Table 1: Identification and explanation of controlled variables which may impact the effect of *C. verum* oil on the growth of *T. aestivum* seeds.

Variable	How It Will Be Controlled	Why It Should be Controlled
The temperature of the Thermoline Scientific Incubator	All petri dishes will be placed in an incubator set to 25˚C.	If the surrounding temperature is above or below 12˚C - 25˚C, which is the optimal temperature for germination of *T. aestivum* seeds, the germination rate may be hindered. This may occur due to a decrease in the seed's moisture levels (Grains Research and Development Corporation, 2016). Therefore varying temperatures may affect the rate of germination.
Number of seeds initially planted in each petri dish	10 *T. aestivum* seeds will be individually counted and then placed into each petri dish.	10 seeds were placed in each petri dish to avoid overcrowding. If this variable is not controlled, a seed's growth potential may not be reached as the seed expends energy to compete for nutrients, space and water rather than into growing (Goodspeed, 2002).
The volume of emulsion applied to each petri dish	A 10mL measuring cylinder will be used to measure out 5mL of emulsion. The emulsion will then be poured into a spray bottle to allow for even application.	A large volume of the emulsion can hinder the growth of *T. aestivum* seeds as it can prevent the seed's roots from accessing oxygen. If this variable is not controlled, the seed's may also lack moisture content and the volume of nutrients required for successful growth (Johnson, 2020).
Growth Medium	Seeds will be grown on cotton wool circles measured out using a 30cm ruler.	Planting mediums have a significant impact on a seed's growth as it provides adequate anchor and support for which the seed requires when growing. Planting mediums also provide aeration and drainage (Mahmoud *et al.*, 2019). Therefore an unsuitable medium can inhibit the growth of a seed.
Species of *Triticum* Seeds Explored	*Triticum aestivum* seeds will be explored. This will be controlled by taking all seeds from the same packet.	Different species of wheat will not always grow the same. This is because light and moisture conditions play a role in a seed's growth and germination (Tomme, 2010). If this variable is not controlled, it may result in incomparable results.

The volume of water sprayed every 2 days	A 10mL measuring cylinder will be used to measure out 2mL of water.	Even though water is required for the growth of all plants, too little water could dry seeds out and too much can oversoak them (Blatt *et al.*, 2014). Therefore if this variable is not controlled the seeds may struggle to germinate.
Time In which the seeds are left to grow	The seeds will be monitored for 6 days after they are initially planted.	If the time in which the seeds are left to grow is not controlled, some seeds would naturally have increased growth.
Spacing of the seeds in the petri dish	A 30cm ruler will be used to measure 1cm from the edge of the petri dish. The seeds will also be approximately 1cm apart.	If seeds are grown close to other seeds they may compete for nutrients, water and space. Therefore seeds may struggle to grow to their full potential as they expend energy in competing for resources rather than growing (Goodspeed, 2002).
Even distribution of water over the seeds	A spray bottle was chosen as the apparatus to water the seeds as it projects similar amounts of water each time the trigger is pulled. The spray bottle was held 10cm from the petri dish to evenly distribute the water.	*T. aestivum* seeds often need to reach a moisture content of 35-45% to begin germination. If this variable is not controlled and the seeds receive too much water or too little water, the rate of germination and growth may be hindered (Grains Research and Development Corporation, 2016).

Uncontrolled:

The health of the seeds: It was assumed that all of the seeds were sufficiently healthy and will show success in germination and growth. However, a seed's health can be dependent on the seed-producing plant they come from. Seeding-producing plants that are strong and disease-free are more likely to produce healthy seeds (Tande, 2019). As seed colour can determine a seed's health, this variable can be minimised by observing the seed's colour before use.

The volume of water in the petri dishes before germination: Despite the fact that a controlled volume of water was initially added to each petri dish, the water may evaporate at different rates because the incubator can heat inconsistently. Therefore the *T. aestivum* seeds may germinate at different times because a moisture content of 35-45% is often required for germination (Grains Research and Development Corporation, 2016). Differing evaporation rates could be minimised if the incubator is maintained at a consistent temperature to assist in equal rates of evaporation.

Materials

- 16 x 90mm Petri Dish
- 16 x Labels
- 300mL Tap Water
- 40mL 100% *Cinnamomum verum* Oil
- 1 x Packet of *Triticum aestivum* Seeds - approximately 200 seeds
- 1 x Scissors
- 9 x 10mL Plastic Measuring Cylinder (±0.5mL)
- 1 x 100mL Plastic Measuring Cylinder (± 0.5mL)
- 9 x 100mL Plastic Spray Bottles
- 1 x Glass Thermometer (±0.5˚C)
- 8 x 100mL Conical Flask (± 12.5mL)
- 8 x Rubber Stopper
- 10 x 1mL Disposable Plastic Pipette (± 0.125mL)
- 2 x Bulb Pipette Filler
- 2 x 1mL Glass volumetric pipette (± 0.05mL)
- 1 x Thermoline Scientific Incubator, set to 25˚C (± 0.5˚C)
- 1 x Calliper (± 0.01mm)
- 1 x 100mL Beaker (± 12.5mL)
- 1 x Fresh Raw Chicken Egg Yolk
- 1 x Glass Stirring Rod
- 1 x Tweezers

Method

Preparation of Different *C. verum* Emulsion

39.9mL of water was measured using both a 100mL measuring cylinder and a 1mL volumetric pipette. 0.1mL of *C. verum* oil was then measured using a second 1mL volumetric pipette. The water and *C. verum* oil were then poured into a 100mL conical flask. Further to this, the yolk of a raw chicken egg was separated from the egg white and placed into a second 100mL beaker. The yolk was stirred using a glass stirring rod and 0.5mL was measured out using a 1mL plastic pipette. It was then added to the same conical flask containing the *C. verum* oil and water. A rubber stopper was added and the flask was swirled in order to thoroughly mix the emulsion (Seal School, 2020). This conical flask was labelled 0.2500% and emulsion A. Concentrations 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500% and 0.2000% were then made using the same method but with the following volumes outlined in Table 2.

Table 2: To investigate the effect of *C. verum* oil on the germination and rate of growth of *T. aestivum* seeds, concentrations 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500% and 0.2000% were made using the following volumes of emulsion A, B and tap water.

Emulsion A (mL)	Tap Water (mL)	Labelled (%)
8.0	2.0	0.2000
6.0	4.0	0.1500
6.0	9.0	0.10 and Emulsion B
2.0	8.0	0.0500
0.1	9.9	0.0025
0.0	10.0	0.0000
Emulsion B (mL)	Tap Water (mL)	Labelled (%)
0.1	9.9	0.0010

Preparing the Petri Dishes

A 30cm ruler was used to approximately measure out circles with a diameter of 9cm and scissors were used to cut them out. Two of these circles were then stacked into each petri dish and, using tweezers, 10 *T. aestivum* seeds were placed on top. Using a permanent marker on the lid of each petri dish, the seeds were labelled with a number from 1-10 and each petri dish was labelled A or B and its assigned concentration of *C. verum* oil (Figure 1).

Spraying the Emulsion

Using a 10mL measuring cylinder, 5mL of the 0.0000% emulsion was measured. This emulsion was then poured into a 100mL spray bottle. The spray bottle was then continuously sprayed until all of the emulsion was evenly projected onto all 10 seeds. This was repeated 7 times for the following emulsion concentrations: 0.0001%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000%, and 0.2500%. Finally, the petri dish lids were placed on top and the petri dishes were placed into the incubator set at 25˚C.

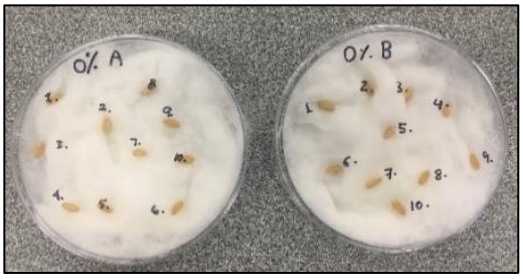

Figure 1 – *T. aestivum* seeds numbered from 1 to 10 on petri dish A and B at 0% *C. verum* oil

Recording the Data

Every 2 days, 2mL of tap water was measured using a 10mL measuring cylinder. To control the application, it was then poured into a spray bottle which was sprayed until all of the water had been projected onto the surface of the cotton wool. Each time the seeds were watered, the lid was placed on top. The seeds were then monitored for 6 days inside the incubator. Each day, the number of seeds that had germinated were recorded (Table 3). Once the seeds had germinated they were removed from the incubator and left on the windowsill to grow. From then, every 2 days a calliper was used to measure the length of the plumule (Table 4).

Risk Assessment

Safety Considerations: Care should be taken when handling glassware including conical flask, volumetric pipettes, beakers, stirring rods and thermometers. Glassware is dangerous because when broken often shatters into sharp pieces. However, this can be prevented by checking glassware for any chips or cracks before use. If glass is to shatter, it should be swept up with a brush and dustpan and then placed into the designated glass disposal bin. Furthermore, as people may be allergic to 100% *C. verum* oil or raw chicken egg, gloves should be worn. Wearing gloves can also assist in minimising contamination. Finally, if an incubator is not wired correctly it can be a possible source of electric shock. To prevent this from occurring, each time the incubator is used the electrical safety should be checked.

Disposal of Chemicals: The *C. verum* oil emulsion should be disposed of in the bin as when cooled may solidify resulting in damage to the drainage system.

Environment and Ethical Disposal of Biological Species: The *T. aestivum* seeds will be disposed of in the bin. This is done to minimise the growth of any unwanted species which may occur if they were to be washed down the sink. As raw egg is a food substance and therefore is likely not to be harmful, it was disposed of down the sink.

Results

Raw Data

Table 3: The number of *T. aestivum* seeds that germinated over 6 days when exposed to varying concentrations of *C. verum* oil

			Concentration (%)							
			0.0000	0.0010	0.0025	0.0500	0.1000	0.1500	0.2000	0.2500
Number of germinated seeds	Day	1	19	20	20	18	0	0	0	0
		2	1	0	0	2	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
		6	0	0	0	0	0	0	0	0

Table 4: The length of the *T. aestivum* seed's plumule when exposed to concentrations 0.000%, 0.0001%, 0.0025% and 0.0050% of *C. verum* oil

			Concentration (%)							
			Day 1				Day 6			
			0.0000	0.0010	0.0025	0.0500	0.0000	0.0010	0.0025	0.0500
(Length of Plumule (±0.01mm)	Petri Dish A	1	10.48	9.72	9.18	3.12	75.66	72.33	79.74	73.64
		2	10.10	9.20	7.55	3.87	35.63	83.65	92.34	48.92
		3	8.72	7.37	6.50	4.41	48.99	90.70	59.83	49.80
		4	5.53	*7.46	4.87	3.59	74.97	*7.84	73.55	31.88
		5	8.39	8.46	9.14	*8.48	64.52	100.29	81.23	*12.29
		6	8.39	9.17	7.90	7.60	76.55	101.82	64.30	68.01
		7	9.03	6.53	9.17	5.94	101.01	78.61	76.85	53.25
		8	4.94	8.99	9.24	*0.00	63.91	91.11	58.54	*34.20
		9	8.88	2.17	6.24	3.85	78.32	32.45	53.15	49.27
		10	6.33	2.67	7.91	2.17	27.78	7.39	78.56	42.93
	Petri Dish B	1	9.10	9.54	6.03	2.92	59.32	96.60	74.93	28.21
		2	10.44	10.60	6.75	*1.65	88.99	83.78	88.06	*0.00
		3	10.16	9.51	9.88	0.00	108.22	86.29	79.64	48.19
		4	8.53	10.76	9.16	5.56	62.93	72.51	78.85	65.92
		5	9.11	11.57	7.31	1.70	90.97	80.66	64.18	12.10
		6	10.04	7.15	7.89	0.00	71.22	69.71	86.63	41.51
		7	10.11	2.91	8.85	4.53	91.41	39.54	70.81	54.02
		8	9.01	10.68	7.44	6.36	90.64	79.06	75.76	76.84
		9	9.83	8.01	8.47	7.81	92.18	79.49	77.23	77.58
		10	7.47	6.53	3.18	6.95	72.31	64.55	60.87	87.81

Legend: The data points marked with * were removed. This was because a human error occurred on the first day where some *T. aestivum* seeds were mishandled, and the plumule became detached from the seed which ultimately affected their potential growth.

Figure 2 – The seed is not attached to the beginning of the plumule but attached to the end

Qualitative Data

- 18/03/21:
  - 0.2000% petri dish B seeds 4 and 5, 0.1500% petri dish B seeds 5 and 6 and 0.1000% petri dish B seeds 2 edges were turning brown.
- 19/03/21:
  - 0.0500% petri dish A seed 8 and petri dish B seed 3 had no plumule indicating that these seeds may not have fully germinated.
  - The plumule of seed 4 (0.0010% petri dish A) and seed 5 (0.0500% petri dish A) was knocked when measuring using the calliper resulting in it detaching from the seed.
  - Despite that the plumule was measured from end to end, its full length may not have been recorded as the calliper could not measure the curved parts.
  - At 1:25 pm the incubator temperature was 26˚C.
  - When the emulsion was sprayed some of it was projected onto the bench surrounding the petri dish.
- 22/03/21:
  - A large volume of water had settled to the bottom of 0.0000% petri dish B.
  - In 0.0500% petri dish A the roots of seed 8 were growing towards the seed (Figure 2).
  - In 0.0010% petri dish A the plumule of seed 4 was very short as it had been damaged in early growth.
- 24/03/21:
  - Of the petri dishes whose seeds had germinated the cotton wool was very dry.
  - In petri dishes 0.0025% A and 0.0500% B mould was growing.
  - The plumule of seed 8 was broken from the seed in petri dish 0.0500% A.

Processed Data

Table 5: The total number of *T. aestivum* seeds germinated over 6 days depending on the concentration of *C. verum* oil and standard deviation for each concentration

	Concentration (%)							
	0.0000	0.0010	0.0025	0.0500	0.1000	0.1500	0.2000	0.2500
Total number of germinated seeds	20	20	20	20	0	0	0	0
Standard deviation	7	7	7	7	0	0	0	0

Table 6: The rate of growth of the *T. aestivum's* seeds plumule over 6 days to investigate the effect of the *C. verum* oil concentration

			Concentration (%)			
			0.0000	0.0010	0.0025	0.0500
[1] Mean length of the plumule (±0.02mm)	Day 1	Petri dish A	8.08	7.14	7.77	4.32
		Petri dish B	9.38	8.73	7.50	3.98
	Day 6	Petri dish A	64.73	73.15	71.81	52.21
		Petri dish B	82.82	75.22	75.70	54.69
[2] Rate of growth of the plumule (%)	Days 1-6	Petri dish A	9.44	11.00	10.67	7.98
		Petri dish B	12.24	11.08	11.37	8.45
[3] Mean rate of growth (%)			10.84	11.04	11.02	8.22
[4] Standard deviation			1.40	0.04	0.35	0.23

Sample Calculations

[1] Mean length of plumule for day 1 of petri dish A at 0.0000% *C. verum* oil concentration

$$Formula: \frac{Total\ sum\ of\ all\ numbers}{Number\ of\ items\ in\ the\ set}$$

$$Mean\ Length\ of\ Plumule: \frac{10.48+10.10+8.72+5.53+8.39+8.39+9.03+4.94+8.88+6.33}{10} = 8.08mm$$

[2] The rate of growth of the plumule for petri dish A over 6 days at 0.0000% *C. verum* oil concentration

$$\text{Formula: } \frac{\text{Mean Final Length} - \text{Mean Initial Length}}{\text{Number of Days of Growth}} \text{x } 100$$

$$\text{Rate of Growth for Petri Dish A: } \frac{64.73-8.08}{6} \text{x } 100 = 9.44\%$$

[3] The mean rate of growth of the plumule for 0.0000% *C. verum* concentration

$$Formula: \frac{Mean\ rate\ of\ growth\ petri\ dish\ A + Mean\ rate\ of\ growth\ petri\ dish\ B}{2}$$

$$\text{Rate of Growth: } \frac{9.44+12.24}{2} = 10.84\%$$

[4] Standard deviation for the mean rate of growth of the plumule for 0.0000% *C. verum* oil concentration

$$\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n}}$$

$$\sigma : \sqrt{\frac{(9.44-10.84)^2+(12.24)^2}{80}} \approx 1.40$$

A Pearson's r Correlation Test was also conducted to determine if there is a statistical difference between the number of germinated *T. aestivum* seeds and the concentration of *C. verum* oil (Mukaka, 2012). Consequently, a null and alternate hypothesis was generated.

Null hypothesis: There is no significant difference in correlation between the following concentrations of *C. verum* oil: 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500% and the number of germinated *T. aestivum* seeds over 6 days; any observed relationship is possibly due to chance or error.
Alternate hypothesis: There is a significant difference in correlation between the following concentrations of *C. verum* oil: 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500% and the number of germinated *T. aestivum* seeds over 6 days; any observed relationship is possibly not due to chance or error.

This statistical test was also conducted to determine if there is a statistical difference between the rate of growth of the *T.aestivum* seed's plumule and the concentration of *C. verum* oil (Mukaka, 2012). Consequently, a second null and alternate hypothesis were generated.

Null hypothesis: There is no significant difference in correlation between the following concentrations of *C. verum* oil: 0.0000%, 0.0010%, 0.0025%, 0.0500% and the rate of growth of the *T. aestivum* seed's plumule; any observed relationship is possibly due to chance or error.
Alternate hypothesis: There is a significant difference in correlation between the concentrations of *C. verum* oil: 0.0000%, 0.0010%, 0.0025% and 0.0500% and the rate of growth of the *T. aestivum* seed's plumule; any observed relationship is possibly not due to chance or error.

$$Pearson's\ r\ Formula: \frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2\sum(y_i-\bar{y})^2}}$$

$r = correlation\ coefficeint$
$x_i = x\ values\ (concentrations\ of\ C.verum\ oil)$
$\bar{x} = mean\ of\ x\ values$
$(x_i - \bar{x})\&(y_i - \bar{y})\ deviation\ value$
$y_i = y\ values\ (rate\ of\ growth\ of\ T.aestivum\ seed's\ plumule\ or\ number\ of\ germinated\ seeds)$
$\bar{y} = mean\ of\ y\ values$
$(x_i - \bar{x})^2\&(y_i - \bar{y})^2\ deviation\ values\ squared$
$\sum(x_i - \bar{x})(y_i - \bar{y})\ product\ of\ deviation\ value$

Table 8: The correlation coefficient between the following pairings: the number of germinated *T. aestivum* seeds and the concentration of *C. verum* oil; and the rate of growth of the plumule and concentration of *C. verum* oil.

	Concentration	F-Critical Value
Number of Seeds Which Germinated	-0.710	-0.285
Rate of Growth	-0.995	-0.159

Note: Excel (2016) graphing package was used to find the correlation coefficient
In biology, a p-value of 0.05 is used. So a level of significance table was used to find the critical r-values (Statistics solution, 2021).

Analysis and Conclusion

Uncertainty: In this experiment, uncertainty occurred when measuring the volume of water and *C. verum* oil. The uncertainty on the 10mL and 100mL measuring cylinders was ± 0.5mL and on the 1mL plastic pipette was ± 0.125mL. There was also ± 0.01mm uncertainty when using the calliper to measure the plumule length. When monitoring the temperature in the incubator with the glass thermometer, the uncertainty was ±0.5˚C and for the incubator itself, the uncertainty was also ± 0.5˚C.

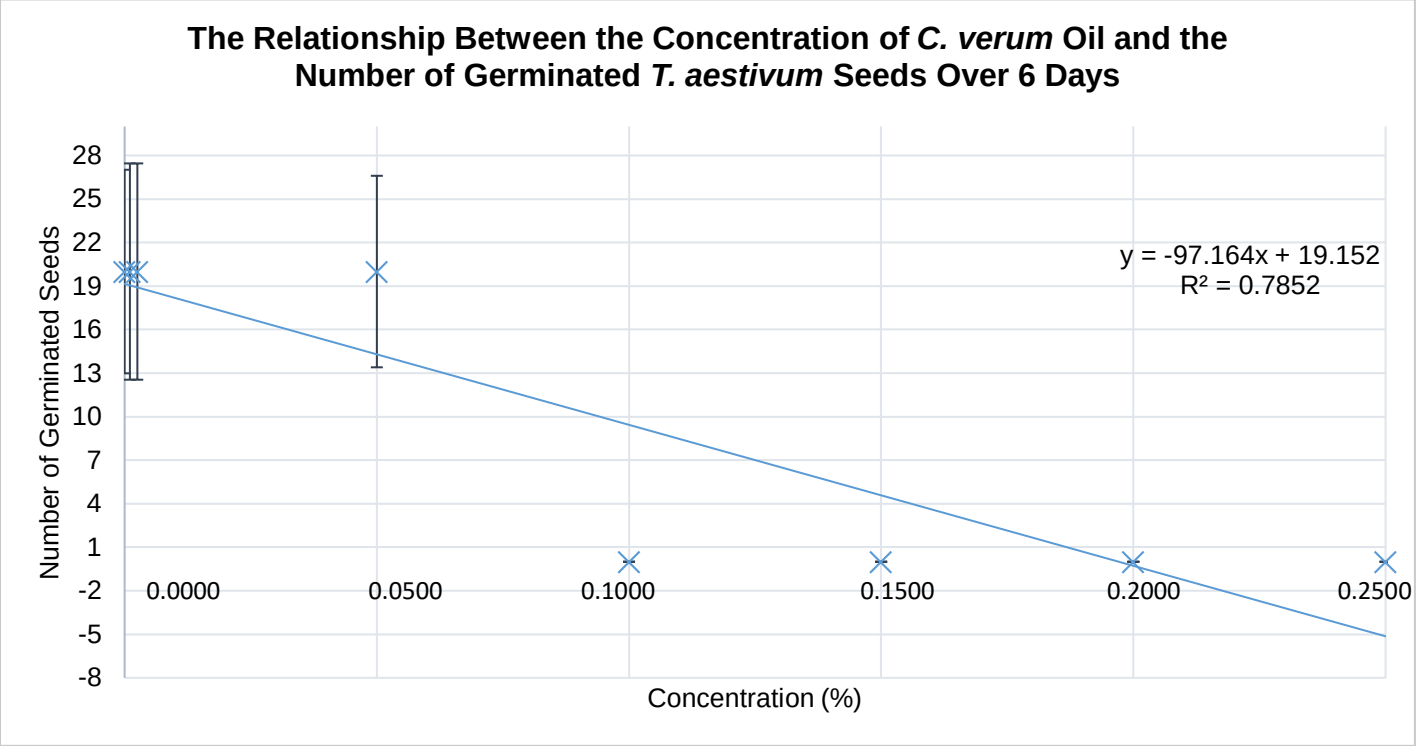

Figure 3 – A scatter plot was drawn to indicate where the number of germinated T.aestivum seeds is dependent on the concentration of C. verum oil

The data in Figure 3 suggests that there is a negative linear trend between the number of germinated *T.aestivum* seeds over 6 days and the concentration of *C. verum* oil. In Figure 3, it can be observed that none of the *T.aestivum* seeds between the concentrations 0.10% and 0.25% germinated, whereas all of the *T.aestivum* seeds between the concentrations 0.00% and 0.05% germinated. The high R^2 value of 0.79 supports that this correlation between the number of *T. aestivum* seeds and the concentration of *C. verum* oil is strong. Further to this, as some of the data points lie close to the line of regression, this suggests the data to some extent is accurate. Furthermore, this correlation is supported by Pearson's r-value. As the r-value of -7.10 is smaller than the r critical value of -0.285, the null hypothesis is accepted and hence the alternate hypothesis is rejected. This may be evident in the data of Figure 3, as the error bars of all concentrations overlap by 7. Thus, this indicates that there is no statistical difference present in this correlation. As the error bars are of moderate size, which reflects the standard deviation, this indicates that multiple random errors may have occurred and that there is likely to be a moderate amount of variability between the data.

The overall correlation found was that higher concentrations of *C. verum* oil are most effective at preventing the germination of *T.aestivum* seeds. This is highlighted by how none of the *T. aestivum* seeds germinated at concentrations of 0.1000%, 0.1500%, 0.2000% and 0.2500%. As *T.aestivum* seeds were used to replace *L. rigidum* seeds, this correlation is supported by the data of an experiment conducted by Campiglia *et al.* (2007). Campiglia's experiment suggested that when *C. verum* oil is at a concentration of 0.0346%, 52% of *L. rigidum* seeds are expected to be inhibited and hence 48% of *L. rigidum* seeds are expected to have germinated. As a small sample was used in this experiment, these exact results could not be observed. However, at 0.2000%, which was slightly lower than the concentration of 0.0346% explored in Campiglia's experiment, it would be expected that many of the seeds would germinate. The data in this experiment supports Campiglia's results as all 20 *T. aestivum* seeds germinated at a concentration of 0.2000%. Furthermore, this correlation is also supported as essential oils which produce allelochemicals can disrupt processes such as photosynthesis, respiration, water and hormonal balance overall affecting germination. Therefore, it is expected that at higher concentrations of *C. verum* oil, where higher amounts of these allelochemicals are present, these processes will be more disrupted and fewer seeds will germinate (Soltys

et al., 2013). This can be observed in the data of Figure 3, as all 20 seeds at 0.05% concentration germinated, whereas none of the seeds at 0.2500% germinated. Therefore as the data of Figure 3 to some extent follows the trends and understanding of scientific research, the data may have a moderate level of accuracy.

In conclusion, as the null hypothesis was accepted, this indicates that there may be no statistical difference in correlation between the number of germinated seeds and the concentration of *C. verum* oil. The data of Figure 3 supports the correlation present as the lowest concentration where all 20 *T. aestivum* seeds did not germinate over the 6 days was 0.0010%. Therefore this may be the most effective concentration of *C. verum* oil. Consequently, as *T. aestivum* seeds were used in replacement of *L. rigidum* seeds when practically applying this to an agricultural situation a small volume of *C. verum* oil would likely be required. This indicates that *C. verum* oil may be an efficient and cost-effective solution to weed growth.

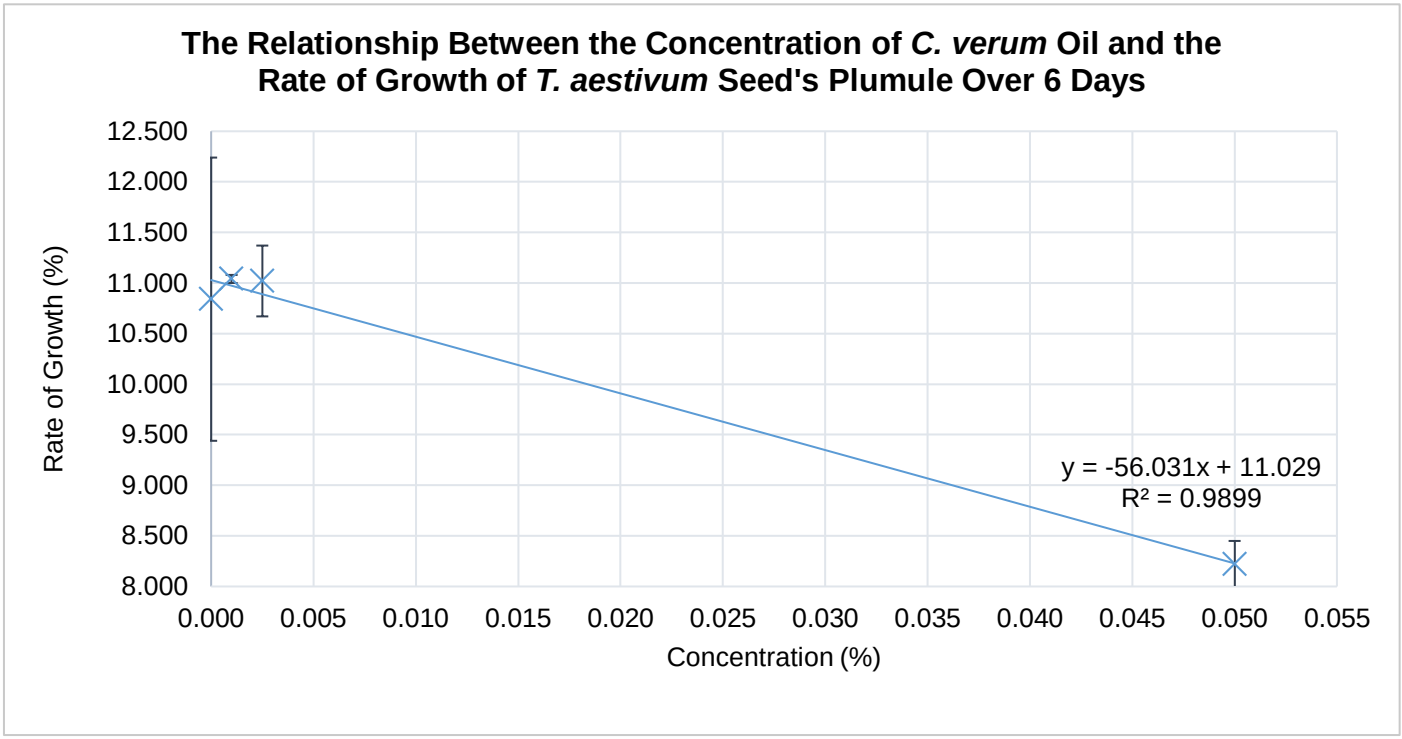

Figure 4 – A scatter plot was drawn to indicate the rate of growth of the *T. aestivum's* seeds plumule dependent on the concentration of *C. verum* oil

Figure 4 suggests that there is a negative linear trend between the rate of growth of *T. aestivum* seed's plumule and the concentration of C. verum oil. The Pearson's r correlation coefficient of –0.995 indicates that there is a very strong relationship between the rate of growth of *T. aestivum* seed's plumule and the concentration of *C. verum* oil (Table 8). As the r-value of -0.995 is smaller than the r-critical value of -0.159, the null hypothesis is accepted and hence the alternate hypothesis is rejected. This is further indicated by how the error bars of 0.0000% overlap with both the error bars of 0.00010% and errors bars of 0.0025% by approximately 0.3500%. Consequently, this suggests that there is no statistical difference present. As the error bars reflect the standard deviation, and they are of moderate size, this indicates that it is likely random errors occurred. The standard deviation also suggests that the values are less reliable as they are not closely clustered around the mean. Furthermore, the high R^2 value of 0.99 supports that a strong negative correlation is present which suggests that the rate of growth of the plumule and concentration of *C. verum* oil is tightly correlated. Table 4 also highlights that a large number of data points were processed to generate the plumule's rate of growth suggesting that moderate accuracy is present as any random events which may affect the data are likely to have been minimised.

The overall correlation found was that higher concentrations of *C. verum* oil were most effective at decreasing the rate of growth of the seed's plumule. This suggests that *C. verum* oil, if not outright preventing weeds species from growing, may be able to stunt a plant's growth. This correlation is supported by scientific research as aromatic essential oils have been shown to emit growth inhibitors which particularly affect plumule elongation. These growth inhibitors include a range of monoterpenoids such as α-Pinene, β-Pinene and camphene which are all found in *C. verum* oil (Abdelwahab *et al.*, 2013). Therefore it is expected that higher concentrations of *C. verum* oil, which naturally would contain higher concentrations of monoterpenoids,

would largely inhibit cell proliferation in the plumule (Dudai *et al.*, 1999). This could be observed as a shorter plumule length. However, when observing the data points more closely the rate of growth of the plumule at 0.0010% concentration (11.04%) was faster than the rate of growth of the plumule at 0.0000% concentration (10.84%). An experiment conducted by Cavalieri and Caporali (2010), proposes that *C. verum* oil at a concentration of 0.0000% inhibits the germination of *Lolium spp* seeds by 70% and at a concentration of 0.001% inhibits the germination of *Lolium spp* seeds by 67%. Therefore this experiment proposes that the germination rate should decrease when concentrations of *C. verum* oil increase. As it would be expected that the plumule growth rate would then decrease when concentration increases, this suggests that part of the data differs from the expected results, indicating some data inaccuracy.

In conclusion, as the null hypothesis was accepted this indicates that there was no significant difference between the 4 concentrations of *C. verum* oil. Therefore 0.0500% concentration of *C. verum* oil may not be more effective at hindering the rate of plumule's growth than the 0.0000% concentration. The data of Figure 4 suggests that the most effective concentration of *C. verum* oil for inhibiting the rate of plumule growth is 0.0000%. As *T. aestivum* seeds were used instead of *L. rigidum* seeds, this indicates that 0.0000% may also be the most effective concentration for inhibiting these seeds' growth. However, only hindering the rate of growth of the *L. rigidum*'s plumule may not be sufficient when practically applying this to an agricultural situation, as the weed species is still growing and competing. Overall, the most effective *C. verum* oil concentration to prevent the germination and growth of *L. rigidum* seeds was 0.1000% (Figure 3).

Evaluation

Strengths and Limitations

- One strength is that 20 seeds were observed for each concentration. Therefore the seeds mean plumule length was calculated from 20 data points. Through multiple trials, the impacts of errors can be reduced and hence aids in the data validity.
- A second strength was that 8 variations of the independent variable were explored. As this decreased the interval between the varying concentrations this caused less uncertainty on the effect of *C. verum* oil and in the process may have increased the data's reliability.
- However, one limitation was *L. rigidum* seeds classification as a weed species which meant *T. aestivum* seeds were chosen instead. Despite these two seeds sharing similar characteristics, they ultimately are different species and therefore this may limit the ability to generalise the results to the wider context. As this is a method to study how *C. verum* oils affect the growth of *L. rigidum* seeds it would not have significantly hindered the reliability or accuracy of the results.
- A second limitation was that only 2 petri dishes, each with 10 seeds, could be explored. This limitation may have significantly impacted the results because if a controlled variable was not relatively controlled in petri dish A, such as the volume of the emulsion was too large, all 10 seeds are likely to be affected. This limitation may result in an insufficient number of replicates to estimate the variability that may be present of *T. aestivum* seeds plumule length (Gouveia, 2017). An improvement would be to factor in more time to count each petri dish, containing 10 *T. aestivum* seeds as one trial, rather than counting each seed.

Errors

Table 9: Explanation of the errors which may have occurred and an improvement for each

Type	Error	Explanation	Direction of Error and Its Effect on the Results	Suggested Improvements
Random	Inconsistent temperature to which the petri dishes were exposed.	Even though the incubators were set to 25˚C, when the room temperature was above the incubator temperature, the incubators' temperature would increase. Therefore the petri dishes most likely would not have consistently been exposed to 25˚C.	Since all seeds were placed in the same incubator, this error may have significantly affected the results. This is because as higher temperatures may stunt a seed's development the germination of all *T. aestivum* seeds would likely have taken longer (Hatfield and Prueger, 2015). This may have resulted in unreliable data indicated by the overlapping of error bars in Figure 4.	A better incubator model which can accommodate for fluctuations in surrounding temperature could be purchased.

Random	Part of the emulsion was sprayed onto the bench surrounding the petri dishes.	Despite that 5mL of the emulsion was measured out using a 10mL measuring cylinder, due to the spray bottle's wide nozzle, part of it ended up on the bench. Therefore petri dishes were likely to receive varying volumes of emulsion.	As water makes up part of the emulsion, this may decrease the volume of water each seed received. If seeds are exposed to varying volumes of water it is likely they will germinate at different times (Grains Research and Development Corporation, 2016). This may have resulted in unreliable data indicated by the overlapping of error bars in Figure 4. However, as only some seeds may have been affected this error is unlikely to largely affect the results.	To aid in equal distribution of solution, the seeds could have been directly dipped into the solution and then using tweezers placed onto the cotton wool.
	Inconsistent determination of when a seed had germinated.	It was assumed that when there was any growth outside of the seed, the seed had begun germinating. However, for some seeds the root may grow first (Panawala, 2017). Therefore the roots may have been mistaken for the plumule.	Some of the petri dishes were removed from the incubators before all seeds had fully germinated (Table 4) resulting in the likelihood that some seeds would have had shorter plumules (Grains Research and Development Corporation, 2016). This may have caused variability suggested through the moderate standard deviation. However, as this error may have affected a small number of seeds it is likely to not have largely affected the results.	To only remove the petri dish once all of the seeds have a thick stem-like structure. This could be checked more regularly such as in the morning and afternoon or a microscope could also be used to observe the cracking of the seed coat.
Systematic	Inaccurate measurement of the plumule length	Even though the plumule was measured from end to end, due to how the plumule only straightens in the later stages of germination, the curved parts may not have been accounted for. (Carrillo-López, 2019).	This could have significantly impacted the results as all of the plumule's length may have been recorded shorter than what they were. This may have led to inaccurate measurements being recorded, suggested by the unclear trend compared to the established scientific work.	Use a flexible ruler or tape measure that allows the curves to be measured.

Extension

An extension to this experiment would be to use the same concentrations of *C. verum* oil but investigate its effect on other widely grown crops. Cavalieri and Caporali (2010) state that the essential oils' selectivity of weed species is yet to be investigated. Therefore, even though this may be an effective herbicide on *L. rigidum*, if *C. verum* oil also kills plants that are of benefit to the agricultural economy, this may hinder its potential in becoming an environmentally friendly solution to target weed growth. *C. verum* oil could be tested on barley or canola as these are two of the main grain crops grown in Australia and therefore are likely to have *L. rigidum* growing nearby (United States Department of Agriculture, 2017).

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505-5099.Westrich.Cinnamon Oil's Effect On Ryegrass Seed Germination

Log Book

Part 1: Proposal of Ideas for IA Experiment

Date: 11th November 2020

Idea	Outline of Methodology Which May Be Used	Thoughts / Notes
What happens after the use-by date of dairy – How much longer after this date is milk still safe to drink?	Explored in different types of milk (eg. soy milk, cow's milk, almond milk, rice milk) Or raw milk vs pasteurised milk Independent variable could be temperature or exposure to contamination (eg. open container vs closed container)	Would need to conduct more research on how to test the effect (eg. how to measure the presence/growth of bacteria) It may take a long time to conduct – would need to factor in time restraints
The effect essential oils may have on inhibiting weed species growth	Create an essential oil and water emulsion to be dripped onto each seed – in addition to water Most likely would be grown in petri dishes to control temperature but could be small trays	What weed species should be used? Can I use weed species or would I need to find an alternate seed as to not add to the already number of weed species growth? Which essential oil? – Have read about peppermint, cinnamon, lavender and clove Would link to my personal experience of growing up in the Barossa Valley which allowed me to observe the large volumes of synthetic herbicides used on crops.

Part 2: Initial IA Scaffold Completed for Approval Which Includes the Initial Research into the Topic Chosen

Date: 1st - 12th February 2021

Research Question/ Aim

- What are you trying to discover or investigate in your lab?
  Aim: To determine the most effective concentration of cinnamon oil (%) at inhibiting the germination and rate of growth (%) of ryegrass seeds, investigated through the use of wheat seeds.

- Must be in the form of a question not answered with yes/no
  How does the concentration of cinnamon oil (0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500%) affect the germination and growth of ryegrass seeds?

Background Information – Introduction

- Discuss the background of your experiment and relevant details

- Global Context:
  - Increased global crop productivity has led to more farms using synthetic fertilisers to kill any weeds that may be of harm to their crops (Dolores and Amparo, 2018).
  - Across Australia weeds impose 5 billion dollars' worth of damage as they cause; costly mechanical and labour measures which reduce weed growth to be implemented, loss of income due to a downgrade in produce quality and space available to grow seeds of practical use. Ryegrass is one of the costliest weeds (Mcleod, 2018).

- Ryegrass
  - Endemic to the temperate regions of Europe and Asia
  - Its high competitiveness and ability to produce an extremely high number of seeds for one plant gives ryegrass its invasive nature (Kloot, 1983).
  - Tufted grass with hairless bright green narrow leaves. When fully grown can produce up to 45,000 seeds in a square metre and hosts the bacteria Clavibacter spp. causing it to be poisonous to livestock (Peltzer, 2020).
  - While it is mostly wind-pollinated it is also able to self-pollinate because of its high levels of self-incompatibility - contributes to its fast-growing nature grass (Chastain, 2013).

- Synthetic herbicides
  - Glyphosate is the most common synthetic herbicide. Due to its overuse, the creation of glyphosate-resistant crops is beginning to form (Yanniccari, 2017).
  - Potential hazards to human health - cause skin irritancy after short-term exposure or nerve axon damage after long-term exposure (Dolores and Amparo, 2018).
  - Can cause damage to the environment - contaminate the groundwater and surface water affecting any surrounding wildlife (U.S. Fish & Wildlife Service, 2009).

- Alternate herbicide: cinnamon oil
  - Essential oils contain natural chemicals called allelochemicals.
  - Allelopathy: the study of how secondary metabolites affect the biological system.
  - Allelochemical compounds are produced by plants, algae, bacteria and fungi and can directly or indirectly release phytotoxic effects to limit competing plant's growth in the surrounding environment (Cavalieri and Caporali, 2010).
  - Essential oils are also able to rapidly decompose in the environment (Cavalieri and Caporali, 2010).
  - Cinnamon oil can control a broad spectrum of weeds. Its vast application increases the likelihood that this essential oil will be used in the future, and therefore this essential oil was chosen to be explored in this experiment (Choi and Hwang, 2013).

Part 3: Risk Assess Submitted on RiskAssess

Date: 12th May 2021

Before I was able to experiment in the laboratory at school a risk assessment had to be generated. Some of the major risks and methods of minimising these risks are as follows:

1) Glassware: care should be taken when handling glassware including conical flasks, volumetric pipettes, beakers, stirring rods and thermometers. Glassware is dangerous because when broken often shatters into sharp pieces. This risk can be minimised by checking glassware for any chips or cracks before use. If glass is to shatter, it should be swept up with a brush and dustpan and then placed into the designated glass disposal bin.
2) Gloves should be worn because people may be allergic to cinnamon oil or raw chicken egg. Wearing gloves also assists in minimising contamination.
3) Incubator: If not wired correctly an incubator can be a possible source of electric shock. To prevent this from occurring, each time the incubator is used, the electrical safety should be checked.
4) Scissors: Sharp points or the blades may cause puncture wounds or cuts. This risk can be minimised by holding the sharp point of the scissors down (by one's side) when moving around the room.
5) Disposal of Chemicals: cinnamon oil emulsion should be disposed of in the bin as when cooled may solidify resulting in damage to the drainage system.
6) Environment and Ethical Disposal of Biological Species: wheat seeds will be disposed of in the bin. This is done to minimise the growth of any unwanted species which may occur if they were to be washed down the sink. As raw egg is a food substance and therefore is likely not to be harmful, it was disposed of down the sink.

505-5099.Westrich.Cinnamon Oil's Effect On Ryegrass Seed Germination

Part 4: First Data Collection – Not Successful

Date: 4th – 10th March 2021

First independent Variable: Concentrations of cinnamon oil: 0%, 1%, 5%, 10%, 14%

Second independent Variable: Type of seed – wheat seed or kangaroo paw seed (native seed)

Dependent Variable: Number of wheat seeds that had germinated over 6 days

- Method: 25 petri dishes with 12 wheat seeds and 25 petri dishes with 12 kangaroo paw seeds were placed in an incubator set at 25˚C for 6 days. There was no germination of any seeds that were sprayed with cinnamon oil concentrations of 1%, 5%, 10% or 14%. All wheat seeds that were sprayed with water only did germinate. None of the kangaroo paw seeds grew.

- Thoughts/Notes: these concentrations of cinnamon oil did not provide sufficient data for this experiment as it only indicated that when wheat or kangaroo paw seeds are exposed to any concentration of cinnamon oil they will not germinate.
- Whereas I was wanting to investigate what approximate concentration of cinnamon oil is most effective at inhibiting weed growth. Although 1% cinnamon oil concentration was effective at inhibiting all wheat seeds from germinating, it could not be observed at what concentration of cinnamon oil some wheat seeds will germinate.

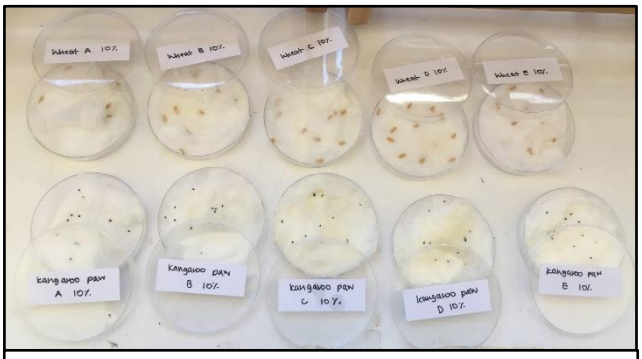

Figure 1 - 10% Wheat + Kangaroo Paw A - B - C - D - E (4th March 2021)

- Upon reflection, it was determined that the concentrations of cinnamon oil were too high and therefore this experiment was repeated.

Part 5: Second Data Collection - Not Successful

Date: 11th – 12th March 2021

Independent Variable: Concentrations of cinnamon oil: 0%, 0.25%, 0.50%, 0.75%, 1%

Dependent Variable: Number of wheat seeds that had germinated over 6 days

- Method: 25 petri dishes with 12 wheat seeds were placed in an incubator set at 25˚C for 6 days. There was no growth in any of the wheat seeds that were sprayed with cinnamon oil concentrations of 0.25%, 0.50%, 0.75% and 1%.
- Thoughts/Notes: these new concentrations of cinnamon oil also did not provide sufficient data from this experiment only as it still only indicated that when wheat seeds are exposed to any concentration of cinnamon oil they will not germinate.
- Kangaroo Paw seeds were not explored in this trial and further trials due to time restrictions. There also was not a time frame large enough to allow them to germinate.
- Upon reflection, it was determined that the concentrations of cinnamon oil were still too high and therefore this experiment was repeated once more.
- Seeds did become mouldy.

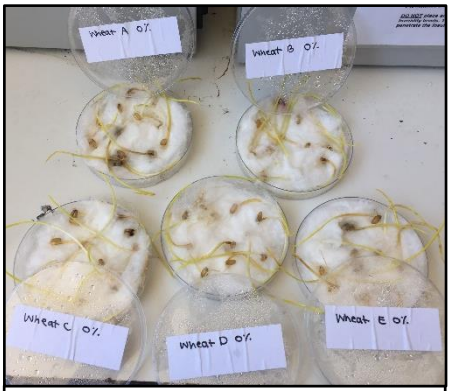

Figure 2 - 0% Wheat A - B - C - D - E (11th March 2021)

505-5099.Westrich.Cinnamon Oil's Effect On Ryegrass Seed Germination

Part 6: Third Data Collection – Successful

Date: 17th – 26th March 2021

Independent: Concentrations of cinnamon oil: 0.0000%, 0.0010%, 0.0025%, 0.0500%, 0.1000%, 0.1500%, 0.2000% and 0.2500%.

Dependent: Number of wheat seeds that have germinated in 6 days and of the germinated seeds, the length of each seed's plumule (mm).

- Method: 18 petri dishes with 12 wheat seeds laid on cotton wool were placed in an incubator set at 25˚C for 6 days. There was no growth in any of the wheat seeds that were sprayed with cinnamon oil concentrations of 0.1000%, 0.2000%, 0.2500% and 0.2500%.

- Thoughts/Notes: these new concentrations of cinnamon oil did provide sufficient data from this experiment. It suggested that the most effective concentration of cinnamon oil at inhibiting the germination and growth of wheat seeds was 0.1%.

Results - Raw Data:

Table 3: The number of wheat seeds that germinated over 6 days when exposed to varying concentrations of cinnamon oil

	Concentration (%)								
			0.0000	0.0010	0.0025	0.0500	0.1000	0.1500	0.2000	0.2500
Number of germinated seeds	Day	1	19	20	20	18	0	0	0	0
		2	1	0	0	2	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
		6	0	0	0	0	0	0	0	0

Table 4: The length of the wheat seed's plumule when exposed to concentrations 0.000%, 0.0001%, 0.0025% and 0.0050% of cinnamon oil

			Concentration (%)							
			Day 1				Day 6			
			0.0000	0.0010	0.0025	0.0500	0.0000	0.0010	0.0025	0.0500
Length of Plumule (±0.01mm)	Petri Dish A	1	10.48	9.72	9.18	3.12	75.66	72.33	79.74	73.64
		2	10.10	9.20	7.55	3.87	35.63	83.65	92.34	48.92
		3	8.72	7.37	6.50	4.41	48.99	90.70	59.83	49.80
		4	5.53	*7.46	4.87	3.59	74.97	*7.84	73.55	31.88
		5	8.39	8.46	9.14	*8.48	64.52	100.29	81.23	*12.29
		6	8.39	9.17	7.90	7.60	76.55	101.82	64.30	68.01
		7	9.03	6.53	9.17	5.94	101.01	78.61	76.85	53.25
		8	4.94	8.99	9.24	*0.00	63.91	91.11	58.54	*34.20
		9	8.88	2.17	6.24	3.85	78.32	32.45	53.15	49.27
		10	6.33	2.67	7.91	2.17	27.78	7.39	78.56	42.93
	Petri Dish B	1	9.10	9.54	6.03	2.92	59.32	96.60	74.93	28.21
		2	10.44	10.60	6.75	*1.65	88.99	83.78	88.06	*0.00
		3	10.16	9.51	9.88	0.00	108.22	86.29	79.64	48.19
		4	8.53	10.76	9.16	5.56	62.93	72.51	78.85	65.92
		5	9.11	11.57	7.31	1.70	90.97	80.66	64.18	12.10
		6	10.04	7.15	7.89	0.00	71.22	69.71	86.63	41.51
		7	10.11	2.91	8.85	4.53	91.41	39.54	70.81	54.02
		8	9.01	10.68	7.44	6.36	90.64	79.06	75.76	76.84
		9	9.83	8.01	8.47	7.81	92.18	79.49	77.23	77.58
		10	7.47	6.53	3.18	6.95	72.31	64.55	60.87	87.81

- Legend: * means that this data point was removed because a human error was made. On the first day of measuring the length of the plumule, the plumule was broken resulting in the seed being separated from the plumule due to rough handling of the seed. Therefore because this may not appropriately reflect the growth of wheat seeds these points were removed.
- As a calliper was used to measure the plumule an uncertainty of ±0.01mm was generated.

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