International Baccalaureate

A study on the effect of smoke water on the germination and growth of Eucalyptus pilularis

Background

Australia is a country where bushfires are commonplace during the summer season, and these fires affect much of Australia's flora. As a by-product of this, numerous native Australian plants that inhabit fire-dependent ecosystems have evolved reproductive strategies to adapt to factors associated with fire. These adaptations that affect their germination can be classified as either physical (derived from the immense heat of the bushfire stimulating a seed to germinate) or chemical (derived from a combination of various chemical elements produced by the smoke that stimulates germination).

Aim

The aim of this biology laboratory experiment is to explore the effects of smoke water, a mixture of water, burnt plants and hay, and its effect on the germination and post germination growth *Eucalyptus pilularis* seeds also known as gumnut or blackbutt, an Australian native plant which predominates in forests that are frequently burned.

Research question

Does smoke water stimulate germination and post germination growth of *Eucalyptus pilularis* seeds compared to de-ionized water?

Prediction

Smoke water will successfully germinate more *Eucalyptus pilularis* than de-ionized water, and thus, as a result of this, the post germination growth of the *Eucalyptus piluiaris* seeds by the smoke water will be more effective. Effectiveness, for this experiment, is defined as the height of the seedling that emerges from the germinated gumnut seed. If the various chemicals, such as phosphorous and nitrogenous compounds found in the smoky remnants of organic matter function as chemical triggers, then *Eucalyptus pilularis* will begin its germination out of its dormant state. These phosphorous and nitrogenous compounds, such as $NaN0_3$, $KN0_3$, NH_4Cl and NH_4N0_3, that are naturally occurring in organic matter, are not found in de-ionized water (Dixon et al. 1995), and hence, smoke water is predicted to germinate a larger number of seeds and grow more after germination than de-ionized water[1].

Method

Preliminary experiment

The gumnut seeds were obtained from trees growing in local forestry plantations.
It was felt necessary to find out if the gumnut seeds would germinate or not.

1. 50 seeds were planted in 5 Petri dishes of potting mixture (10 seeds per dish).
2. Each dish was watered with 10 ml of de-ionised water and left for two weeks at room temperature.
3. At the end of the two weeks the numbers of seeds germinating was counted.

Results

Number of seeds germinating = 22/50
Percentage germination = 44%
The supply of seeds was considered viable enough to proceed with the experiment.

[1] http://anpsa.org.au/APOL2/jun96-6.html

Equipment

- 10 Petri dishes
- 100g of "Yates premium quality" potting mix
- 5.00g of hay
- 5.00g of Eucalyptus leaves
- 5.00g of grass
- Electronic weighing scale (±0.01g)
- 100 seeds of *E. pilularis* that are 2.00 mm in diameter (±0.5mm)
- 10.0cm ruler (±0.5mm)
- 100ml of de-ionized water to create the smoke water
- 100ml of de-ionized water to create the control
- Tea strainer
- 3 x 250ml graduated beaker (±0.4mL)
- Matches
- 2 Sand baths
- 2 thermometers (±0.05°c)

To create the smoke water

1. Place 5g each of the hay, grass and Eucalyptus leaves into one of the 250ml beaker.
2. Ignite the organic matter with a match so that they catch on fire. Let them burn until they are all charred.
3. Measure 100ml of de-ionized water with the second 250ml beakers. Pour this water into the first beaker with the leaves, hay and twigs and leave to infuse for 5 hours.
4. Strain the smoke water mixture into the third measuring beaker using the tea strainer, ensuring that you are only left with the liquid remnants.
   SAFETY Care should be taken when burning the organic matter, this should be carried out in a ventilated area and the beakers should be made of heat resistance glass.

Germination and growth

1. Set the sand baths to 30 degrees Celsius and place a thermometer in each one to verify the temperature setting.
2. Place 5 Petri dishes into one sand bath and the remaining 5 Petri dishes into another. One will be our control and one will be our test.
3. Measure out 10 x 10.0g of the potting mix using the electronic weighing scale and place 10.0g into each one of 10 Petri dishes. 5 dishes for smoke water treatment and 5 dishes for de-ionised water treatment.
4. Sow 10 gumnuts into each Petri dish and submerge them into the potting mix at a consistent depth of 0.5cm. Place the seeds towards the edges of the Petri dish so they can be observed through the glass without having to disturb the seeds to observe them.
5. Water the control sand bath at 8:15am with 10ml of de-ionized or smoke water each day for fourteen days.
6. After 14 days, count the number of seeds germinated (distinguished by the emergence of the seedling) and measure the height of the emergent seedling in the test and the control groups with the 10.0cm ruler. The seedling height is measured from the soil surface to the highest part of the stem.
7. Repeat the set up once to ensure sufficient data.

Controlled Variables

- The same volume (10ml) of liquid is added to each dish at the same time (8:15am) each day throughout the 14 days.
- All 100 *E. pilularis* seeds that were used in this experiment were kept within a size range of 2.00 mm in diameter
- The water used to create the smoke water was de-ionized water like the control, which allowed consistency between the control and the test groups.

- The temperature of the seeds was kept constant at 30.0°C by the sand baths.
- The potting mix for the seeds was from the same brand, "Yates premium potting mix" and the mass of potting mix used for the seeds was kept constant at 10.0g.
- Same amount of light was assumed to be received for each plant as the experiment was conducted in the same location on the same days.
- The seeds were placed at a depth of 0.5cm into the soil in the Petri dish.

The experiment continued for fourteen days to allow for sufficient time to gauge of the effect of the different water types, the manipulated variable. Both sand baths set at the same temperature are placed next to each other, as specified by the method, and they are assumed to be receiving equal amounts of light. The potting mix was taken from the same batch, so all samples could be assumed to contain the same ratio of ingredients. Furthermore, the *E. pilularis* was submerged into the potting mix at a consistent depth of 0.5cm and towards the edges of the Petri dish to allow for observations to be made through the glass without having to disrupt the seeds to observe them.

Our method of data collection for this experiment is to count the seeds that successfully germinated from the different Petri dishes in the control and test groups respectively, the measured variable. This is done by observing through the side of the Petri dish whether the seed coat has broken and the seedling has emerged. The other way to collect data in this experiment is to measure the height of the seedlings (from the soil surface to the seedling tip) of the germinated seeds after the 14 days of the experiment. The difference between smoke water and de-ionised water was determined using the χ^2 test for the germination and the t-test for the growth of the seedlings.

Assumptions

- The light is of the same intensity because the seeds will be set up side by side.
- The de-ionized water contains the same impurities
- The potting mix contains the same amount of its constituent components.
- The impurities and chemical elements in the air will be the same for both sets of seeds.
- The gumnut seeds are all composed of the same percentage of elements.

Observations

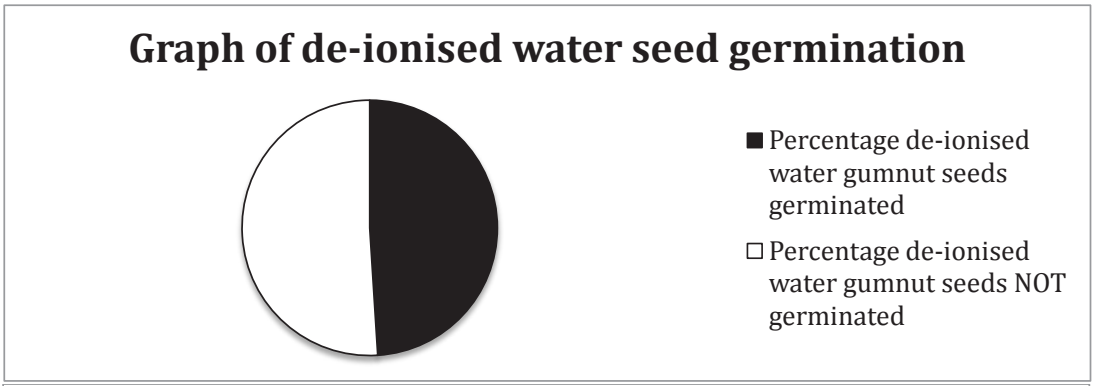
- The *E. pilularis* seeds were no bigger than 2mm, and were brownish black in colour. There were no obvious signs of previous germination, or cracking of the outer seed coat.
- The smoke water was clearly distinctive from the de-ionized water. The de-ionized water was clear, as one would expect if it had been filtered. The smoke water, however, had a blackish, straw coloured hue, due to its absorption of the remnants of the burnt organic matter.
- Definite germination was seen on a lot more seeds with the smoke water than with the de-ionized water.
- The *E. pilularis* subjected to smoke water germinated earlier on average than the seeds subjected to de-ionized water. Seeds with smoke water started showing first signs of germination as early as 7 days, when their seed coats started to split to allow the seedlings to emerge. In comparison, the de-ionized watered seeds took up to 10 days to start showing germination.
- The *E. pilularis* that were germinated by the smoke water tended to have larger seedlings emerging from the split seed coat.
- The *E. pilularis* that were watered with the smoke water had significantly larger cracking of the seed coat, allowing for more space for the seedlings to grow and extend outwards from the shell.
- The colour of the seedlings in both experiments was a distinct dark purple colour, and leaves appeared only on the smoke water experiment, with a maximum of 2 small, juvenile leaves found, measuring no more than approximately 50.0mm.

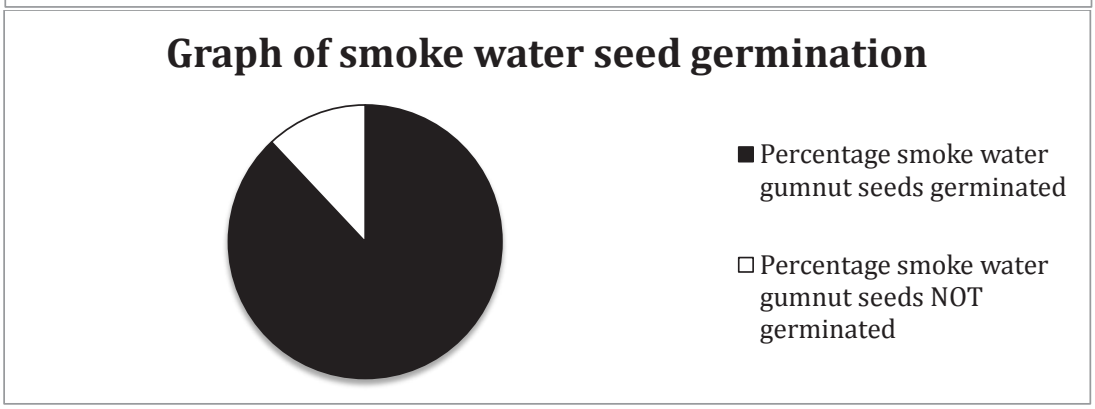
Number of seeds successfully germinated

In order to determine the number of seeds that were germinated successfully, the number of seeds that showed distinct cracking of the seed coat and the emergence of the seedling for both the smoke water and the de-ionized water test groups were counted and placed into the table below. The raw data is presented in appendix A.

Water Type	Trial	Numbers germinated (/50)	Average	%
De-ionized	1	26	25	49
	2	23		
Smoked	1	43	44	88
	2	45		

From the processed data that informs us about the number of seeds successfully germinated, we can clearly see that smoke water germinates, on average.





X^2 test

In order to see if there is a significant difference between the germination of the seeds treated with smoke water and de-ionised water a X^2 test was carried out.

Null Hypothesis: Smoke water does not affect germination of gumnut seeds
Alternative Hypothesis: Smoke water affects germination of gumnut seeds

	Smoke water	De-ionised water	Row total
Germinated	88	49	137
Not germinated	12	51	63
Column total	100	100	200

Proportion of seed germinating = 137/200 = 68.5%
Proportion of seeds not germinating = 100 – 68.5 = 31.5%

Expected number of smoke water treated seeds to germinate = 68.5% of 100 = 68.5
Expected number of de-ionised water treated seeds to germinate = 68.5% of 100 = 68.5
Expected number of smoke water treated seeds not to germination = 31.5% of 100 = 31.5
Expected number of de-ionised water treated seeds not to germinate = 31.5% of 100 = 31.5

Observed frequency	Expected frequency	Difference	Positive difference	
O	E	O-E	IO-EI	$(IO-EI)^2/E$
88	**68.5**	**19.5**	19.5	**5.55**
49	**68.5**	**-19.5**	19.5	**5.55**
12	**31.5**	**-19.5**	19.5	**12.07**
51	**31.5**	**19.5**	19.5	**12.07**
			X^2_{calc}	**35.25**

Number of degrees of freedom = (rows – 1) x (columns – 1) = (2-1) x (2-1) = 1

X^2_{crit} = 3.84 for p=0.05

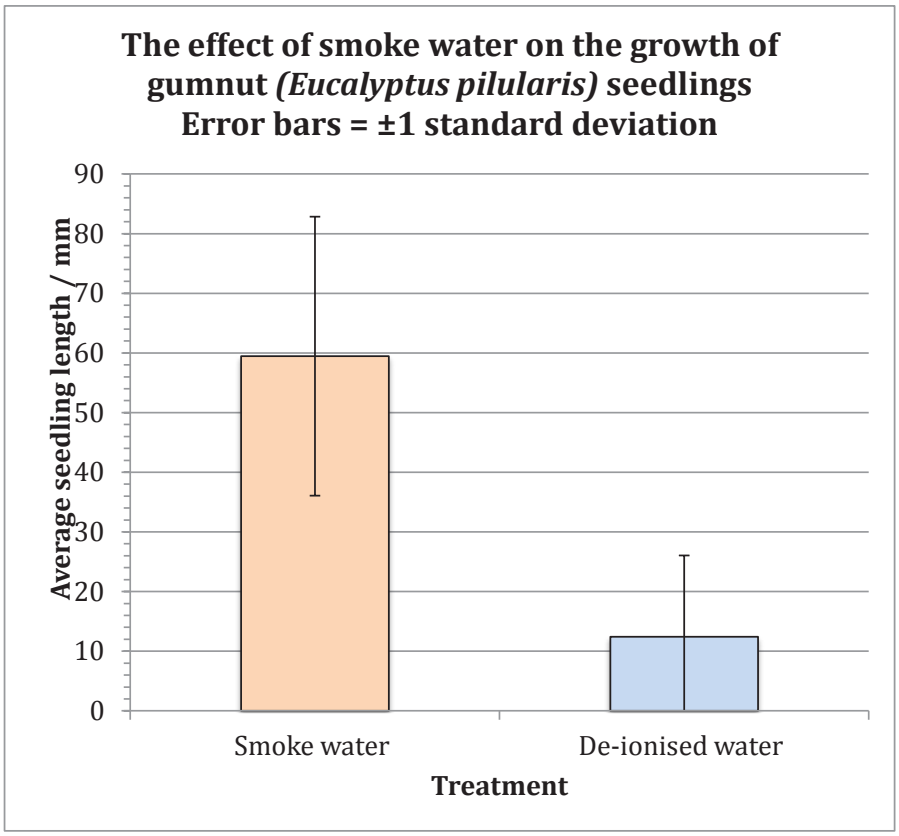
Since the test value for X^2_{calc} = 35.25 is a lot greater than the critical value X^2_{crit} = 3.84 we must reject the Null Hypothesis and accept the Alternative Hypothesis. The test value is significant for $p < 0.001$

The effect of smoke water and de-ionized water on post germination growth

This section of the experiment is designed to test the effectiveness of gumnut seed germination, depending on the type of water it received, either de-ionized or smoke water. Effectiveness was determined by the height of the seedling that emerged from the seed coat of the germinated gumnut seeds. The higher the seedling the more effective the water is on germination. The raw data is presented in appendix A.

Height of seedlings for germinated seeds					
Water Type	**Trial**	**Trial average of seedling height /mm ±0.5mm**	**Trial Standard Deviation**	**Overall average height /mm ±0.5mm**	**Overall standard deviation**
De-ionized	1	13.0	13.4	**23.4**	**13.6**
	2	11.8	13.9		
Smoked	1	57.8	24.5	**59.5**	**12.4**
	2	61.1	22.3		

On first observation of the processed data, it can be seen that smoked water clearly has a higher average seedling height than the de-ionized water whilst also having a lower standard deviation. This indicated that the smoked water seeds seedling grew higher than the de-ionized water. The error bars in the graph below suggest that there may be a significant difference between the affects of the treatment on seedling growth. However, the range of variation in the results as given by the standard deviations is large especially for the de-ionised water treatment trials. To verify this, a t-test was carried out on the data.



t-test

In order to statistically test whether the shoot of smoke water germinated gumnut seedlings grew more than the de-ionized water, a two-tailed t-test for independent samples was carried out to investigate whether there is a significant difference between the growth of the seedlings.

- **Null Hypothesis** - the smoke water has no effect on post germination growth of the gumnut seedlings.
- **Alternative Hypothesis** - the smoke water does have an effect on post germination growth of the gumnut seedlings.

t-test formula:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

degrees of freedom = $\mathbf{n_1 + n_2 - 1 = 198}$
$\mathbf{t_{calc}}$ = **17.4**
$\mathbf{t_{crit}}$ **(p=0.05) = 1.97**

Because our test t value t_{calc} = 17.4 is greater than the critical value t_{crit} =1.97 at p = 0.05, we can accept the alternative hypothesis, that the smoke water significantly stimulates the growth of the gumnut seedlings germinated. The test value is significant for p < 0.001

Evaluation of Weaknesses with suggested improvements

The potting mixture used was obtained from the local garden shop, and whilst the same brand and the same amount of the potting mixture was used for both seeds in the experiment, the potting mixture may have contained impurities which could potentially have enhanced or reduced the ability of the seeds to germinate, especially because the Yates brand "Contains trace elements to add extra vital nutrients"[2]. Some of the chemicals from the smoke water also could have potentially reacted with some of the ingredients of the potting mix and rendered them useless, however the seeds watered with de-ionized water may not have had this potential problem. To improve this, I could have used a different support for the seeds such as cotton wool or filter paper.

Using different types of leaves, twigs and hay to create the smoke water would give you different chemicals, as each has a differing composition of chemicals, some of which may be beneficial for germination, and some of which wouldn't. For this experiment, I could have used only one variable like hay, instead of twigs and leaves as well. This would narrow my scope of results down as well and I would potentially be able to pinpoint the specific chemical, or source of the chemical, that allows gumnuts to germinate successfully. It may be found that twigs, for example, don't enhance seed germination but leaves do. By singling out the element that best enhances seed germination, further experiments could be carried out, and the exact chemical could be identified, that best enhances the seeds germination.

Combined with this, I could have used gumnut seeds that were all the same weight rather than the same size in diameter. I tried to use gumnut seeds that were only 2.00mm in diameter, however it would have been better served to use seeds that all had a constant weight of 0.2g for example, as then I could have assumed that each seed contained the same amounts and composition of nutrients, enzymes and other chemicals inside it.

To further narrow my scope of the experiment, I could have tested the effects of different concentrations of the smoke water as well. Instead of only using a 1:10 ratio of 1 part twigs, hay and leaves to 10 parts de-ionized water, I could have tested a ratio of 1:5 with 1 part twigs, hay and leaves and 5 parts de- ionized water. Working out the optimum concentration of smoke water would help this experiment as better and clearer results could be obtained.

[2] http://www.yates.com.au/products/pots-and-potting-mix/all-purpose-potting-mix/yates-premium-potting-mix/

Conclusion

In conclusion, the experiment supported my hypothesis that smoke water will successfully germinate more *Eucalyptus pilularis* than de-ionized water. Furthermore, the subsequent growth of the *Eucalyptus pilularis* seeds by the smoke water was found to be more effective than the de-ionized water due to the significantly taller seedlings of the *Eucalyptus pilularis* that were exposed to the smoke water. This could because the various chemicals, such as phosphorous and nitrogenous compounds found in the smoky remnants of the burnt organic matter (in my case, the burnt leaves, hay and twigs) acted as chemical triggers for the *E. pilularis* to begin its germination out of its dormant state and stimulate its subsequent growth. While all of the active compounds in smoke have not yet been identified, a large majority of the compounds present in the smoke water mixture ($NaN0_3$, $KN0_3$, NH_4Cl and NH_4N0_3) are water soluble, thus they are easily able to be taken in by the gumnut seed and, once inside the seed, they are used as these so called "chemical triggers" to start germination. These chemical triggers work by altering the levels of chemicals that the seed maintains in homeostasis, once the seed has registered these differing levels of phosphorous and nitrogenous compounds, it stimulates the germination of the seed. There are, however, compounds called *butenolides* that have confirmed germination-promoting action. These *butenolides* are produced by some plants on exposure to high temperatures and smoke caused by bush fires. In particular, botanists Flematti, Ghisalberti, Dixon and Trengove isolated a particular *butenolide* called 3-methyl-2H-furo[2,3-c]pyran-2-one, which was found to trigger seed germination in plants whose reproduction is fire-dependent, such as the *E. pilularis* used in my experiment[3]. One theory about how this *butenolide called* 3-methyl-2tf-furo[2,3-c]pyran-2-one is formed by the plant is given to us by Light, Berger and van Steden, who hypothesized that this particular *butenolide* was created from cellulose within the plant, and this substance, created by the cellulose, stimulated the seeds reproductive cycle, and hence, germination[4]. The two pie graphs that show the percentage of seeds germinated for the smoke water experiment and de-ionized water experiment respectively, furthermore indicate that my hypothesis was correct, with 88% of the smoke watered seeds successfully germinating compared to only 47% of the de-ionized water seeds germinating. This was backed up with my χ^2-test that accurately concluded that we could reject the null hypothesis, with a 95% degree of confidence, that the smoke water successfully germinated more seeds that the de-ionized water. The t-test on the seedling growth shows that the smoke water has a significant positive effect on the gumnut seedlings.

Bibliography

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Marnie E. Light, Barend V. Burger and Johannes van Staden Formation of a Seed Germination Promoter from Carbohydrates and Amino Acids http://pubs.acs.org/doi/abs/10.1021/jf050710u *J. Agric. Food Chem.*, 2005, *53* (15), pp 5936–5942 Publication Date (Web): July 1, 2005

[3] http://www.sciencemag.org/content/305/5686/977
[4] http//pubs.acs.org/doi/abs/10.1021/jf050710u

Appendix A - raw data tables

Seeds watered with Smoke Water (Trial 1)

Seed Number	Did the seed Germinate	Height of seedling in / mm ±0.5mm
1	Yes	56.0
2	Yes	71.0
3	Yes	73.0
4	Yes	67.0
5	Yes	54.0
6	No	0
7	Yes	58.0
8	Yes	70.0
9	Yes	66.0
10	Yes	61.0
11	Yes	64.0
12	Yes	71.0
13	No	0
14	No	0
15	Yes	59.0
16	Yes	67.0
17	Yes	58.0
18	Yes	63.0
19	Yes	62.0
20	Yes	64.0
21	Yes	72.0
22	Yes	75.0
23	No	0.0
24	Yes	68.0
25	Yes	64.0
26	Yes	69.0
27	Yes	70.0
28	No	0
29	Yes	52.0
30	No	0
31	Yes	79.0
32	Yes	81.0
33	Yes	83.0
34	Yes	74.0
35	Yes	74.0
36	Yes	78.0
37	Yes	63.0
38	Yes	69.0
39	Yes	58.0
40	Yes	70.0
41	Yes	68.0
42	Yes	62.0
43	Yes	63.0
44	Yes	68.0
45	Yes	58.0
46	Yes	81.0
47	Yes	68.0
48	Yes	73.0
49	Yes	67.0
50	No	0

Seeds watered with De-ionized water (Trial 1)

Seed Number	Did the seed Germinate	Height of seedling in / mm ±0.5mm
1	Yes	18
2	Yes	27.0
3	Yes	19.0
4	No	0
5	No	0
6	No	0
7	Yes	24.0
8	No	0
9	Yes	25.0
10	No	0
11	Yes	28.0
12	No	0
13	No	0
14	Yes	17.0
15	Yes	23.0
16	No	0
17	Yes	16.0
18	No	0
19	Yes	26.0
20	Yes	27.0
21	Yes	15.0
22	No	0
23	No	0
24	Yes	27.0
25	No	0
26	Yes	21.0
27	Yes	22.0
28	No	0
29	Yes	27.0
30	Yes	37.0
31	No	0
32	No	0
33	Yes	26.0
34	Yes	31.0
35	No	0
36	No	0
37	Yes	27.0
38	Yes	41.0
39	No	0
40	No	0
41	No	0
42	Yes	25.0
43	No	0
44	Yes	19.0
45	No	0
46	No	0
47	Yes	37.0
48	Yes	22.0
49	No	0
50	Yes	25.0

Seeds watered with Smoke water (Trial 2)		
Seed Number	Did the seed Germinate	Height of seedling in mm / ±0.5mm
1	Yes	72.0
2	Yes	73.0
3	No	0
4	Yes	72.0
5	Yes	57.0
6	Yes	74.0
7	Yes	79.0
8	Yes	62.0
9	Yes	78.0
10	Yes	64.0
11	Yes	72.0
12	Yes	79.0
13	Yes	72.0
14	Yes	57.0
15	Yes	56.0
16	Yes	83.0
17	Yes	63.0
18	No	0
19	Yes	72.0
20	Yes	63.0
21	No	0
22	Yes	58.0
23	Yes	81.0
24	Yes	57.0
25	Yes	62.0
26	No	0
27	Yes	74.0
28	Yes	73.0
29	Yes	83.0
30	Yes	58.0
31	Yes	74.0
32	Yes	57.0
33	Yes	63.0
34	Yes	79.0
35	Yes	60.0
36	Yes	74.0
37	Yes	79.0
38	Yes	57.0
39	Yes	86.0
40	Yes	53.0
41	Yes	56.0
42	Yes	67.0
43	Yes	63.0
44	Yes	68.0
45	Yes	54.0
46	Yes	68.0
47	Yes	68.0
48	No	0
49	Yes	62.0
50	Yes	72.0

Seeds watered with De-Ionized water (Trial 2)

Seed Number	Did the seed Germinate	Height of seedling in / mm ±0.5mm
1	No	0
2	Yes	26.0
3	Yes	21.0
4	Yes	23.0
5	No	0
6	No	0
7	No	0
8	No	0
9	Yes	31.0
10	No	0
11	Yes	14.0
12	No	0
13	No	0
14	Yes	16.0
15	Yes	18.0
16	No	0
17	No	0
18	No	0
19	Yes	26.0
20	Yes	31.0
21	Yes	25.0
22	No	0
23	No	0
24	Yes	21.0
25	No	0
26	Yes	31.0
27	Yes	26.0
28	No	0
29	Yes	23.0
30	Yes	36.0
31	No	0
32	No	0
33	Yes	14.0
34	Yes	23.0
35	No	0
36	No	0
37	Yes	23.0
38	Yes	27.0
39	No	0
40	No	0
41	No	0
42	Yes	24.0
43	No	0
44	Yes	45.0
45	No	0
46	No	0
47	Yes	42.0
48	Yes	23.0
49	No	0
50	No	0

12