SAINT JOSEPH'S INSTITUTION INTERNATIONAL

How does increasing the duration (0 to 8 hours) of UV-B irradiation (the process of which a substance is exposed to radiation) affect the success of Vigna radiata germination?

HL Biology Internal Assessment

Joseph Ong

11/1/2017

Research Question

How does increasing the duration (0 to 8 hours) of UV-B irradiation (the process of which a substance is exposed to radiation) affect the success of *Vigna radiata* germination?

Background Information

Vigna radiata, also known as mung beans, is a food source that is consumed daily in dishes such as protein powders, around the world. When I attended a Model United Nations conference, one of the important issues we discussed was the usages of Chlorofluorocarbon (CFCs) fertilizers. The Australian delegate reminded all of us that the hole in the ozone layer- previously more evident in Australia- has resulted in the unfortunate consequence of slower seedling growth. This had me interested as the terms "ozone layer depletion", while often mentioned many, including myself, do not actually know about its relevance in maintaining Earth's ecosystems- since the IB syllabus only mentions greenhouse gases when mentioning atmospheric pollution. Ozone, the gas that comprises majority of the ozone layer, is a molecule that shields Earth from all "UV-A (320 to 400nm wavelengths)" and some "UV-B (290 to 320nm) wavelengths" (Skorucak, 2013). However, with the increased usage of CFCs in India, the nation that I represented at the conference, the Australian delegate informed us that another hole in the ozone layer has become more and more evident in the atmosphere above the Indian nation. CFCs are organic compounds which contain halogen molecules, during combustion these are released into the atmosphere. The increase in chloride radicles, released by CFCs, have resulted in the breakdown of ozone into oxygen under UV light (Brown & Ford, 2014). This lack of ozone molecules increases UV wavelengths ability to enter Earth's atmosphere. With increased amounts of UV-B irradiation entering India, I wanted to investigate whether or not this would have any effect on mung bean germination (a common crop that is grown in India and is also accessible to me). This research is essential, as any effects on production could have a delirious effect on many communities.

Aim

Before researching I assumed that UV-B irradiation would simply cook the beans; however, recent research implies that the production of gibberellic acid ($C_{19}H_{22}O_6$) – a hormone stimulating plant growth (Gupta, 2013)- was inhibited by UV-B irradiation from the sun (Sugimoto, 2013). Based on research from the World Applied Science Journal, prolonged irradiation to "UV-B irradiation can deleteriously affect physiological processes" involved in the germination process (Peykarestan et al, 2012). As a result, this investigation will explore the effects of increased irradiation to UV-B light will subsequently have on the percentage of *Vigna radiata*, which germinated. However, UV-B irradiation duration will not exceed 8 hours due to safety precautions as advised by the (Allot, et al, 2014.)

Hypothesis

It is hypothesise that UV-B irradiation will inhibit the germination process of *Vigna radiata*. This will result in a lower overall percentage of beans that successfully germinate- as the duration of UV-B irradiation increases. Within the context of this experiment the definition of "germination" will be a bean showing evidence of a protruding plumule, a sign of successful bean germination, from the seed radicle. However, the synthesis of "gibberellic acids and other gibberellin hormones" (Allot et al, 2014) will be inhibited due to the UV-B irradiation denaturing the biosynthetic enzymes- such as amylase (Allot et al, 2014)- and various other phenolic compounds required to produce many plant physiological structures essential for germination (Bing et al, 2013). Only with this hormone, can mitosis and starch synthesis occur, which are processes required for germination.

Methodology

Figure 1- Showing the germination process of mung beans

Ong, 2016

Preliminary trials: The following method was adapted from (Royal Queen Seeds, 2016).

The beans used, were obtained from the local market. Therefore, preliminary experiments were required to see if they would germinate, or not. The method is as follows:

1. Place 500 beans into five different petri dishes- 100 beans per dish.
2. Soak the beans in $15cm^3$ of distilled water for one hour.
3. Wrap *Vigna radiata* beans in 30g of moist cotton wool.
4. Count the germination successes of the *Vigna radiata*

Results:

Number of germinated= 78/ 100

Percentage germinated= 78%

These results make *Vigna radiata* seeds viable for experimentation.

Having observed the changes of bean germination at 12, 22 and 44 hours, the relatively small changes in bean germination percentage was deemed insignificant beyond 22 hours. Since the success of germination is the dependent variable, and UV-B irradiation duration was the independent variable, counting the number of germinated bean is a sufficient method to measure bean germination success.

However, the beans were heating up (reference qualitative analysis section), and considered that it might affect the results; the main adaptation of the original method devised was the placing a water sink by placing $10cm^3$ of water on a transparent lid. The 44 hour data set is required for the sole purpose of investigating whether UV-B irradiation permanently inhibits, or whether it merely delays germination- given that no significant changes was noted in the control (the one with no irradiation to UV-B wavelengths).

Independent variable

Time of UV-B irradiation (h)/±0.02

Dependent variable

Percentage success of *Vigna radiata* germination (%)

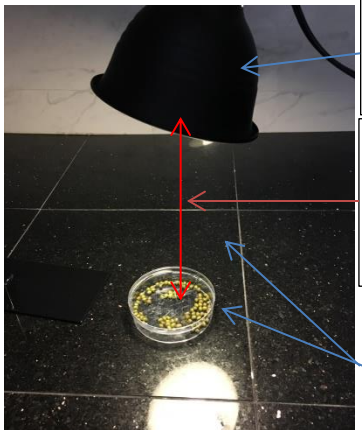
Controlled Variables:

- Temperature (30°C) and humidity (70%) at which beans germinate, was kept constant by a sand bath. This is to ensure that enzyme, and other proteins activity were not affected by any other factor- besides UV-B irradiation duration. Also, the conditions are deemed optimum conditions for germination (Royal Queen Seeds, 2016)
- Brand of *Vigna radiata* was "Nature's Choice". This means that beans are more similar, genetically, to one another- minimising genetic variations that could make it less likely to germinate.
- Same volume of distilled water was used ($20cm^3$); to ensure that the same amounts of energy was absorbed by it, and prevented from reaching the seeds.
- Distance from lamp, 15cm, to prevent varying concentrations of irradiation.
- Location of irradiation. The experiment was conducted in the same dark room, with same bulb. The location of germination was the same laboratory. This was done in order to minimize factors such as O_2 concentration variation- an essential gas for seed germination.

Apparatus and Justification:

1. 9 Petri dishes- to grow beans in
2. 4500 *Vigna radiata* seeds- for experimental repeats.
3. UV-B lamp- to irradiate *Vigna radiata*
4. Transparent lid- to contain water volume (used as heat sink).
5. Sand bath- to keep germination conditions constant.
6. 50cm^3 Measuring cylinder/cm^3 (±0.5) - to add consistent water volumes to germination process.

Figure 2- Showing the UV-B irradiation process



Ong, 2016

Figure 3- Sand Bath apparatus

Royal Queen Seeds, 2016

How to Irradiate the *Vigna radiata*:

1. Place 100 *Vigna radiata* seeds into a petri dish (each seed are one experimental repeat).
2. Provide a heat sink for the experiment; this is done by adding 20cm^3 of water onto a transparent, inverted lid on top of the experimental site.
3. Ensure to place petri dishes exactly 15cm away from the light source prior to switching on the UV light.

Germination methodology:

1. Repeat the procedures of the preliminary trials with hourly intervals- 0 to 8 hours.
2. Leave beans in a 30 °C sand bath throughout the germination process.
3. Ensure to count the number of successful bean germinations after 22, and 44 hours of UV-B irradiation. The 44 hour data set is required for the sole purpose of investigating whether UV-B irradiation permanently inhibits, or whether it merely delays germination- given that no significant changes was noted in the control (the one with no irradiation to UV-B wavelengths).
4. Repeat the experiment 5 times- for all UV-B irradiation time durations to ensure sufficient data is obtained.
5. Use Pearson's correlation coefficient to determine the strength of association between the variables.

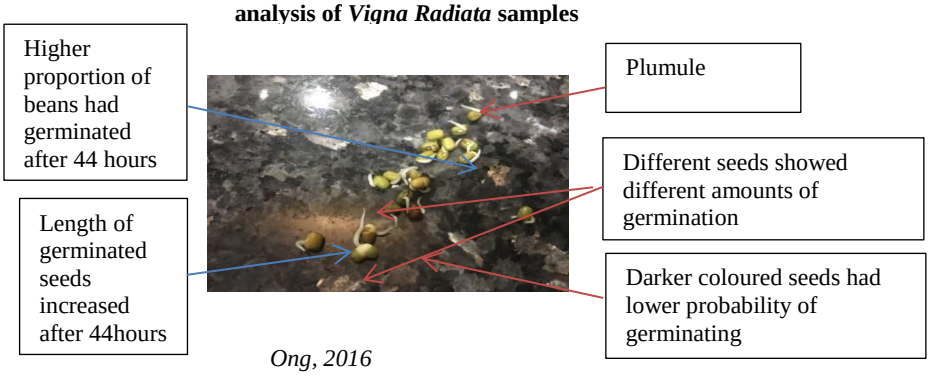
Analysis

Qualitative analysis:

- The length of the plumule seemed longer as the duration of UV-B irradiation increased. A measurement, measured using a ruler, of 10 random samples proved that on average 44 hour samples were longer.
- Despite precautions beans with longer irradiation inevitably heated up slightly more, proven with greater thermometer readings of the water sink above the experimental area- after experimentation.
- Darker colour (dormant beans) generally had a larger failure rate of germination.
- Some of the cotton wool sheets appeared dry after 22 hours.

- Majority of seeds have a shedding testa with an evident plumule.
- Some plumules were detached from any seeds.

Figure 4- Photograph qualitative analysis of *Vigna Radiata* samples



Ong, 2016

Risk Assessment

Safety:

- Wear UV goggles when exposed to light, to prevent eyes swelling.
- Wear a lab coat- prevents UV-burn and skin cancer

Ethical:

- No ethical concerns were involved in this experiment

Environmental:

- Dispose of all radioactive beans, those irradiated with UV-B, into a chemical waste bin. \

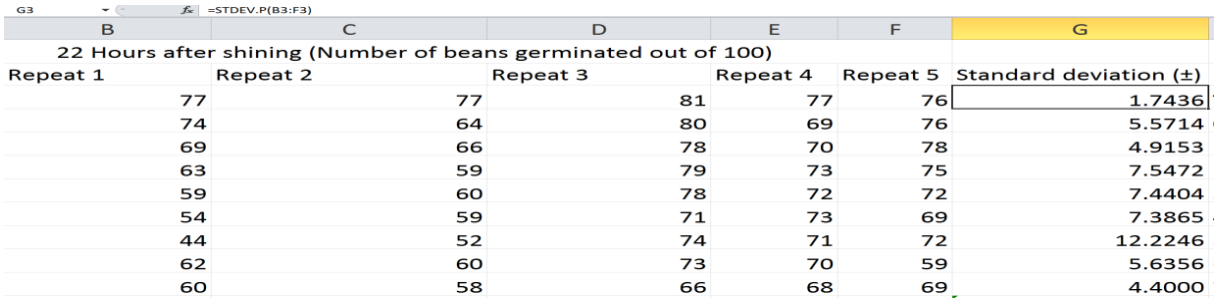
Raw Data tables:

- In order to determine the success of germination, seed germination was determined by the percentage of successfully germinated beans, having undergone different durations of irradiation. The raw and processed data is presented below.

Table of results after 22 Hours of irradiation process (Number of beans germinated out of 100)					
Duration of UV-B irradiation /h(±0.02)	Repeat 1	Repeat 2	Repeat 3	Repeat 4	Repeat 5
0	77	77	81	77	76
1	74	64	80	69	76
2	69	66	78	70	78
3	63	59	79/31	73	75
4	59	60	78	72	72
5	54	59	71	73	69
6	44	52	74	71	72
7	62/ 24	60	73	70	59
8	60	58	66	68	69

Table of results after 44 Hours of irradiation (Number of beans germinated out of 100) (same beans as those inspected after 22 hours)					
Duration of UV-B irradiation /h(±0.02)	Repeat 1	Repeat 2	Repeat 3	Repeat 4	Repeat 5
0	86	84	84	86	81
1	86	85	86	87	84
2	84	81	83	85	85
3	81	79	84	88	83
4	89	82	83	88	84
5	85	84	86	88	84
6	88	83	88	85	81
7	85	87	82	90	82
8	86	86	89	79	82

Figure 5- How to calculate standard deviation for data sets, using Excel.



Anomalies:

At 7 hours repeat one and 3 hours repeat three anomalies in data were recorded. These two anomalies were significantly less than the standard deviation values, and thus were omitted from the final results (they are highlighted in yellow), and the UV-B irradiation durations were subsequently repeated. These values were 24 and 31 despite an average of 64.8 and 69.8 for those time durations.

However, the number of germinated beans was significantly less. As observed, from qualitative analysis section, these two had cotton wool particularly lacking in moisture, thus meaning that insufficient water was present for germination (lack of osmosis activity as no water potential gradient is established).

Example Calculations:

Average=$\frac{Total\ of\ all\ repeats}{Number\ of\ all\ repeats}$ (Buchman et al, 2012)

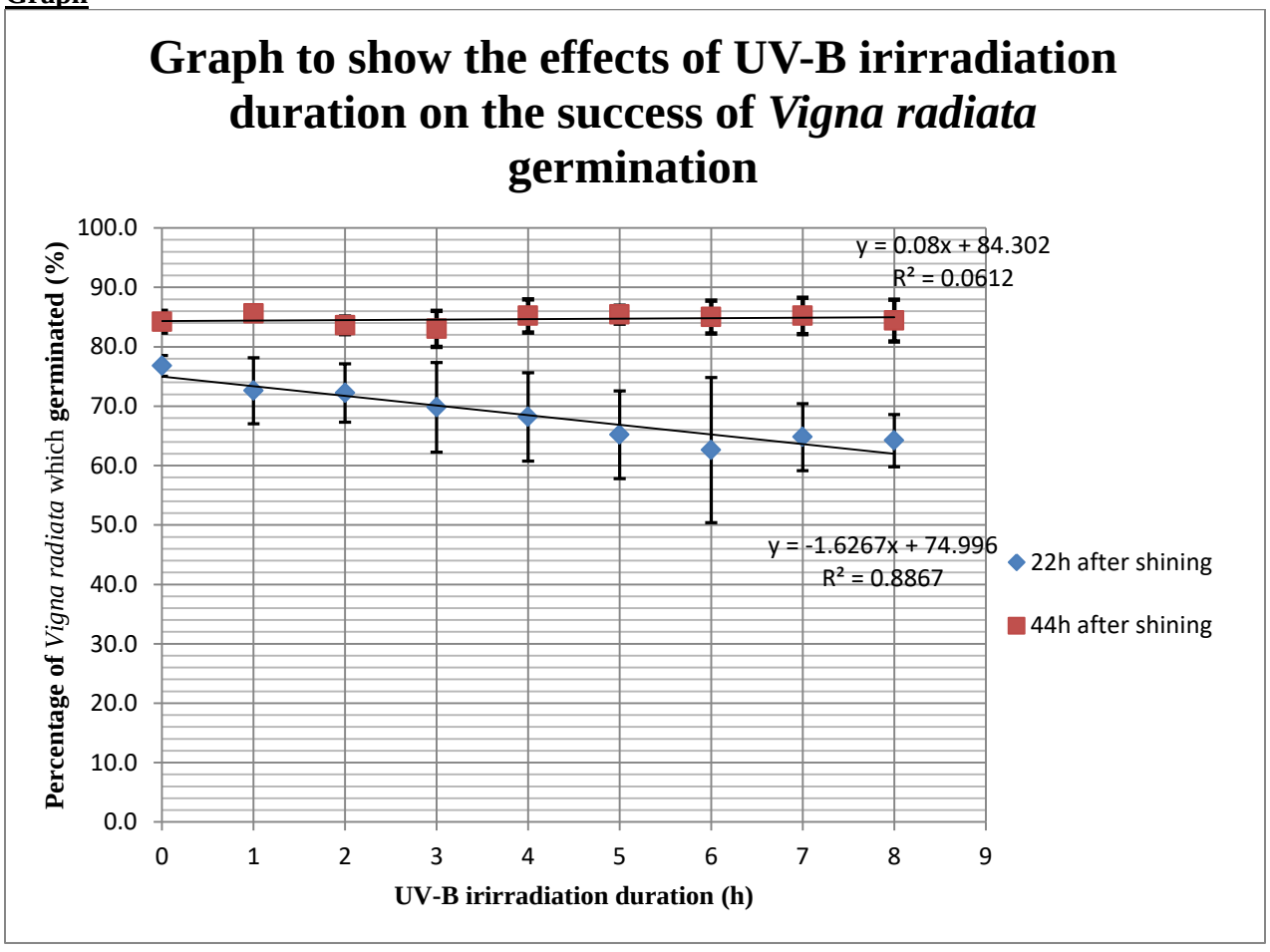
e.g. at 1 hour after 22 hours of irradiation: $\frac{86+84+84+86+81}{5}$= 76.8

Convert all the average values into percentages by dividing 100 (number of repeats per petri dish) before multiplying 100%.

Processed data table showing successful germination after irradiation

Average Percentages of Successful Germination		
Time of UV-B irradiation	Average germination successes after 22h after irradiation (%)	Average germination successes after 44h after irradiation (%)
0	76.8	84.2
1	72.6	85.6
2	72.2	83.6
3	69.8	83.0
4	68.2	85.2
5	65.2	85.4
6	62.6	85.0
7	64.8	85.2
8	64.2	84.2

Graph



Error bars represent ±1 standard deviations. The calculations procedures are shown in Figure 5.

Statistical Tests:

Pearson's Product-Moment Correlation:

This statistical test is a method of measuring the strength of correlation between an independent and a dependent variable (Laerd, 2013). By drawing a line of best fit within the data the Pearson Correlation Coefficient, or r-value, shows the discrepancies between the obtained data sets and the line of best fit. Since this investigation seeks to determine the relationship between changing of UV-B irradiation duration, and the subsequent percentage germination of *Vigna radiata*, the most suitable test is the Pearson's Product-Moment Correlation.

The coefficient obtained will be within the range of -1 to +1. Should the coefficient be negative, as expected, the correlation is negative, and the closer to -1 the value means the stronger the negative correlation. This would mean that a relationship, negative correlation, can be established between the duration of UV-B irradiation, and the subsequent percentage of *Vigna radiata* which germinated.

Equation of Pearson's Product-Moment value:

$r = \frac{N\sum xy - \sum xy}{\sqrt{[(N\sum x^2 - \sum x^2)(N\sum y^2 - \sum y^2)]}}$ (Laerd, 2013)

r = Pearson's Product-Moment Coefficient.
N = Number of each data set, per variable.
$\sum xy$ = Sum of the data sets of the independent and dependent variables
$\sum x$ = Sum of x values
$\sum y$ = Sum of y values
$\sum x^2$ = Sum of x^2 values
$\sum y^2$ = Sum of y^2 values

Table showing Pearson's Coefficient values (22 hours after irradiation)

	UV-B Duration (h)	Percentage germination (%)	IV mean	DV Mean	$N\sum xy - \sum xy$	$N\sum x^2 - \sum x^2$	$N\sum y^2 - \sum y^2$
	0	76.80	-4.00	8.31	-33.24	16.00	1105.19
	1	72.60	-3.00	4.11	-12.33	9.00	152.11
	2	72.20	-2.00	3.71	-7.42	4.00	55.09
	3	69.80	-1.00	1.31	-1.31	1.00	1.72
	4	68.20	0.00	-0.29	0.00	0.00	0.00
	5	65.20	1.00	-3.29	-3.29	1.00	10.82
	6	62.60	2.00	-5.89	-11.78	4.00	138.72
	7	64.80	3.00	-3.69	-11.07	9.00	122.47
	8	64.20	4.00	-4.29	-17.16	16.00	294.31
Mean	4.00	68.49			-97.60	Total= 60.00	Total= 1880.43
						r-value=	-0.29

Table showing Pearson's coefficient calculations (44 hours after irradiation)							
	UV-B Duration (h)	Percentage germination	X mean	Y mean	$N\sum xy - \sum x \sum y$	$N\sum x^2 - \sum x^2$	$N\sum y^2 - \sum y^2$
	0	84.2	-4.00	-0.42	1.69	16.00	2.85
	1	85.6	-3.00	0.98	-2.93	9.00	8.60
	2	83.6	-2.00	-1.02	2.04	4.00	4.18
	3	83.0	-1.00	-1.62	1.62	1.00	2.63
	4	85.2	0.00	0.58	0.00	0.00	0.00
	5	85.4	1.00	0.78	0.78	1.00	0.60
	6	85.0	2.00	0.38	0.76	4.00	0.57
	7	85.2	3.00	0.58	1.73	9.00	3.00
	8	84.4	4.00	-0.22	-0.89	16.00	0.79
Mean	4	86.6		Total	4.80	60.00	23.24
						r value	0.13

Table showing the strength of associations based on obtained values		
	Coefficient, r	
Strength of Association	Positive	Negative
Small	0.1 to .3	-0.1 to -0.3
Medium	0.3 to .5	-0.3 to -0.5
Large	0.5 to 1.0	-0.5 to -1.0

The coefficient value is in this range

Adapted from (Laerd Statistics, 2013)

The r value of this investigation is in the category, as presented in the tables (above). This means that based on this statistical test; the correlation between UV-B irradiation duration and the percentage of *Vigna radiata* germination is a weak negative one. The -0.29 coefficient value, at 22 hours, means that a weak negative correlation exists (reference the table above) between UV-B irradiation duration and the subsequent percentage of *Vigna radiata* germination. However, after 44 hours this weak negative correlation becomes a weak positive correlation as the success of germination increases with time. However, both of these coefficients are too low to be considered definitive answers to the research question. Therefore, secondary data shall be referenced as well (reference conclusion below).
The statistical test did not factor in the overlapping error bars, which indicate extreme variation in the data; it is only with more repeats can anomalies be identified- making the data more reliable. At the seventh and eighth hours,

Uncertainty: Despite evident outlier in data at 6 hours experimental data; that particular data set has a relatively high uncertainty value- as demonstrated by its standard deviation value of 12.2. The high uncertainty for the data set mentioned above means that the anomaly could be due to genetic variance- which would explain the higher standard deviations (at 6 hours). The expected value after 6 hours of irradiation- followed by 22 hours of being placed in moist cotton wool- is 65%. This is well within the range of uncertainty meaning that all experimental uncertainties and could potentially follow the overall trend.

Discussion:

After 22 hours, a downward trend between within the results was observed. This was further evidenced with a negative gradient of the line of best fit of -1.67. However, after 44 hours the trend was no longer evident. A weak positive association was instead calculated using the same statistical test, and although the gradient of the line of best fit was less steep than its 22 hour counterpart, it still implies a positive trend of the data sets; although the 0.0612 (almost 0) gradient at 44 hours- as well as the extremely low 0.13 Pearson coefficient value and overlapping error bars- making these trends inconclusive.

Other graphical observations include a plateau after 5 hours of UV-B irradiation. Since the maximum percentage of *Vigna radiata* germination is at 0 hours, of UV-B irradiation, it can be concluded that no UV-B irradiation is the optimum conditions for the seeds to germinate. Also, after 44 hours, the maximum amount of *Vigna radiata* germination is at 8 hours, of UV-B irradiation, at 84.2% (the same as without any UV-B irradiation).

Conclusion:

In conclusion, this hypothesis is mostly supported by experimental data, and this data is sufficient to answer the research question, **"the duration of UV-B irradiation reduces the percentage success of *Vigna radiata* germination"**. The statement can mostly be applied to the 22 hours after irradiation data sets. A better hypothesis would be- "the duration of UV-B irradiation delays germination in *Vigna radiata* samples" (given that the 44 hour data of the same beans showed minimal differences with prolonged UV-B irradiation). The high R^2 value of 0.89 means- the graphed points are relatively close to the plotted line of best fit. This also means that the line of best fit proving that the data sets is "more representative" of the points plotted (NC State University, 2004). This therefore demonstrated a clear inhibition of proteins- including enzymes such as amylase (A.Allot et al, 2014) - based on the obtained results. However, 44 hours after irradiation, minimal changes in the dependent variable were recorded with changes in the independent variable. Since minimal data changes were recorded, 44 hours after irradiation, when UV-B irradiation duration increased, it can therefore be concluded that seed germination is "mostly delayed" by UV-B irradiation, as opposed to inhibited. Due to safety precautions a relatively weak 60W UV-B lamp was used for irradiation. As a result, the protein synthesis of biosynthetic enzymes- including amylase (A.Allot et al, 2014)- and subsequent production of gibberellic acids might have only been delayed, or only small amounts were denatured. After 44 hours it would appear that since many samples germinated were close to the control; UV-B irradiation potentially damaged a small proportion of the proteins mentioned above, yet not enough to have significant long-term effects.

It can be concluded that increasing UV-B irradiation duration has a significant effect on the germination success of *Vigna radiata*- at least 22 hours after irradiation. The maximum germination of the species is 76.8% while the minimum was at 62.6%, after 22 hours after irradiation. These changes were observed after 0 and 6 hours of UV-B irradiation- 22 hours after irradiation. The 18.5% decline, in percentage germination, solidifies the statement "UV-B irradiation delays germination"- since minimal change was observed 44 hours after irradiation.

Figure 6- Results of *Vigna Mungo* germination (secondary

Treatments UV-B exposure	Final germination (%)	Germination velocity (GV)
0 (control)	94.7a ± 1.5	28.4
10 min	895a ± 2.5	32.7
20 min	81.5b ± 2.8	33.3
30 min	73.3c ± 3.5	23.2
40 min	70.5c ± 2.7	20.5

Mean ± 1 SE. Means not followed by the same letter are significantly different from each other at p≤0.05. Each mean is of five replicates

Adapted from the Effect of enhanced UV-B irradiation on germination, seedling growth and biochemical responses of Vigna mungo (L.)", S. Shahid, M. Farooq, M. Siddiqui, S. Zaidi, 2013

Past studies have shown that results (data shown in Figure 6 to the left) have shown similar results in regards to the effect of UV-B irradiation on bean germination. Despite the different beans used in the study, they are genetically similar as they are of the same *Vigna* genus. Given that the results of (S.Shahdid et al, 2013) were similar to mine; it can be further substantiated that UV-B irradiation has an

effect on percentage germination- based on both experimental findings (reference figure 6).
However, despite the obtained results being in accordance with other published data (reference Figure 6), anomalies were present. As mentioned earlier, the anomalies in my study are rather insignificant. This is due to experimental standard deviations caused by biological variance (addressed in evaluation).

The standard deviations allow for any anomalies in the data to be justified, due to the ranges of experimental values. This is significant because anomalous looking data sets do not need to be omitted- since they could fit into the overall trend. Despite the deviations, the high R^2 value of 0.8867, a number relatively close to 1, it can also be concluded that the data is well represented by the graphed line of best fit. This is in contrast with the low R^2 value of the after 44 hours after irradiation data sets which have an R^2 value of merely 0.0612- a poorly represented line of best fit.

Strengths of investigation

Table showing the strengths of the following investigation	
Strength:	**Justification:**
Large numbers of repeats were conducted (totaling 4500 *Vigna radiata* samples).	This ensures that data collected is representative of the species, and has anomalies that can be easily identified.
A large range of intervals was used (9 intervals).	This ensures that statistical tests e.g. Pearson's Product-Moment correlation, requiring a minimum range of 8 could be used.
The question as to whether or not beans had germinated	Makes it less bias- when determining whether or not the bean had germinated.
Tightly controlled variables. Such as the same water volumes were used to soak, insulate and germinate beans.	This ensures that the germination is not affected by water volume or heat absorbance of *Vigna radiata*.
Acceptable uncertainty within experimental apparatus	Experimental uncertainty can be attributed to biological variance- not the negligible apparatus uncertainty.

Table showing the weaknesses, significance and subsequent improvements of the investigation		
Weakness	**Significance**	**Improvement**
Genetic variance across seeds	Since this was not factored in, it could have led to a large proportion of the experimental uncertainty. More browner seeds would fail to germinate than the lighter coloured ones- large significance.	Ensure to only use the green beans, with similar size and shape during experimentation. This will ensure that beans have minimal genetic variations and that it would not become a cause for the differences in germination successes.
Dispersion of UV-B waves	Large significance- The UV-B energy waves were not concentrated in one area (the experimental area). This could mean that the delay in germination, as opposed to the permanent inhibition, of *Vigna radiata* was due to a lack of UV-B energy.	Either provide a container to contain the UV-B waves or put the lamp closer to the beans to minimize dispersion. Also, as a precaution add more water to the heat sink to prevent heating of beans, have a fire extinguisher nearby for safety purposes.
Usage of cotton wool, with an uneven distribution.	This weakness effects the water absorbency based on how thin the layer is as well as the rate of evaporation. This should have little significance as water should have been in excess. Medium significance.	Use a brand of cotton wool with sheets and ensure that the thickness of the germination site is two sheets thick.
Water loss to surroundings	Despite being covered in cotton wool with a petri dish lid on top of it, and did as observed, causing bean germination to decrease. This, depending on volume of water evaporated, could have had a large effect on final germination percentage. Medium significance.	Ensure to add a dry layer of cotton wool on top of the germinating *Vigna radiata*, preventing water from evaporating without limiting Oxygen availability. This is to ensure that bean germination was not affected by lack of water.
Location of beans on petri dishes	Different water volumes, in a particular area, could influence *Vigna radiata* germination.	Germinate in groups of 10 not 100. This will ensure that more water is provided to each bean and it is easier to ensure that all beans get a location on the cotton wool with appropriate moisture.
Accidental snapping of seeds	This meant some beans which had actually germinated, were deemed non-germinated as there was no evident plumule. Relatively low significance to data obtained as this was not very common.	Look for alternative signs of germination- e.g. the peeling of the seed testa.

Further investigations:

The effects different wavelengths have on seed germination e.g. UV-A and UV-C could be an area for further investigation. Furthermore, the intensity/ concentration of UV irradiation could also be changed- and its effects studied. This is because the break-down of the ozone layer allows for a larger range of frequencies to enter the Earth- affecting the biology of organic molecules. Furthermore, the effects of UV-B exposure times can also be investigated on different growing processes. These include growth (measuring plumule length), dry mass (by evaporating water and weighing) and rate of photosynthesis (by measuring rate of water uptake). This would truly determine the effects of UV-B exposure duration on *Vigna radiata*.

Bibliography:

A.Skorucak, 2013, What is the wavelength of UVa, UVb, and UVc light measured in nanometers, and frequency (in Hz), http://www.physlink.com/education/askexperts/ae300.cfm, <accessed 23rd November 2016>

A.Allot, D.mindorff, 2014, Biology Course Companion, Oxford University press

B. Peykarestan, M. Seify, M.Fadaei and M. Hatim, 2012, pages 11&12, UV Irradiation Effects on Seed Germination and Growth, Protein Content, Peroxidase and Protease Activity in Portulaca grandiflora and Portulaca oleracea, http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.389.9542&rep=rep1&type=pdf , <accessed 20th November 2016>

Bing Liua, Xiao-bing Liua, Yan-Sheng Lia, S.J. Herbertc, 2013, page 2, Effects of enhanced UV-B irradiation on seed growth characteristics and yield components in soybean, http://www.sciencedirect.com/science/article/pii/S0378429013002761, Science Direct, <accessed 8th December 2016>.

C.Brown, M.Ford, 2014, page 194, "Higher Level Chemistry 2nd Edition", Pearson's Education Limited, Edinburgh.

Department of Agriculture, Forestry and Fisheries, 2010, Mung Bean Production Line, page 3, Department of Agriculture, Forestry and Fisheries South Africa.

J.Ong, 2016 Methodology and Qualitative analysis photographs.

Kazuhiro Sugimoto "Seed germination under UV-B irradiation" Minamikyushu University (2013) http://www.nankyudai.ac.jp/library/pdf/43a_1.pdf accessed 11th November 2016, <accessed November 12th 2016>

Laerd Statistics, Pearson Product-Moment Correlation, 2013, Lund Research, https://statistics.laerd.com/statistical-guides/pearson-correlation-coefficient-statistical-guide.php , <accessed 10th December 2016>

L.Buchanan, J.Fensom, E.Kemp, P.Rondie, J.Stevens, pages 572, 2012, Mathematics Standard Level, Oxford University Press, United Kingdom

NC State University, "Graping with excel", 2004, National Science Foundation, https://www.ncsu.edu/labwrite/res/gt/gt-reg-home.html , <accessed 11th December 2016>

R.Gupta, 2013, Gibberellic acid in plants, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4002599/, National Institutes of Health, United States <accessed 6th February 2017>

Royal Queen seeds, 2016 "Germinating seeds", https://www.royalqueenseeds.com/content/45-germinating, United Kingdom, <accessed 12th November 2016>

S. Shahid, M. Farooq, M. Siddiqui, S. Zaidi, 2013, "Effect of enhanced UV-B irradiation on germination, seedling growth and biochemical responses of *Vigna mungo* (L.)" Hepper, page 781, University of Karachi, Pakistan

Tevini, M. & Teramura, A.H. 1989 UV-B effects on terrestrial plants, Photochem. Photobiol., 50, 479–487. Wiley Online Library | CAS | Web of Science®