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TITLE : COLORS OF LIGHT (WAVELENGTH) ABSORBED BY GREEN PLANT
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INTRODUCTION

Plant contains photosynthetic materials which are responsible in the photosynthesis processes. One of the materials is chloroplast. Chloroplast is actually an organelle found in plant cells and many other eukaryotic organisms. The chloroplast, in green plant, contains many pigments; a few of them are chlorophyll a and chlorophyll b, carotene and xanthophylls. All of these are natural pigments. Natural pigments[1], are substances presence in animal and plant cell that produces color due to specific absorption of light and reflection of the unabsorbed light.

In this experiment, the chlorophyll of the plant is going to be extracted and is centrifuged before it is tested with a set of calorimeter. Calorimeter is a device that is used to measure the absorption percentage of wavelength by a substance; in liquid state.

RESEARCH QUESTION

How does different green plant differ in the absorption of wavelengths?

AIM

To investigate the amount of absorption between two green plants (Spinach and Mustard) exposed to different range of wavelengths (440nm, 470nm, 490nm, 520nm, 550nm, 580nm, 590nm and 680nm).

HYPOTHESIS

The rate of absorption of between the two green plants (Mustard and spinach) is different. It is hypothesized that both spinach and mustard will have a large absorption of red and blue colour. Green is of an exception. It is also hypothesized that the mustard will have a lower absorption of green colour than that of spinach. Spinach has a greener color due to the existence of larger volume of chlorophyll than that that is available in the mustard. Hence it imposes a greener colour due to a higher reflection of green colour.

VARIABLES

Variables		Method to control	Range
Independent Variable	The wavelengths that are imparted on the green plant form a calorimeter	Different wavelengths represents different colours are varied by using a calorimeter. This will ensure that the contents of the extracted pigments will be subjected to different colours.	440nm, 470nm, 490nm, 520nm, 550nm, 580nm 590nm and 680 nm
Dependent Variable	The amount of absorption of light by the extracted pigments.	The value of light absorbed is measured from a calorimeter. The calorimeter will register the result of the absorption of light corresponded to the wavelengths.	-
Controlled Variable		How to control	
Type of organisms used		The experiment is carried out by using the same organism in the experiment which is plant organisms. Different organisms may not have the materials needed to absorb the wavelength.	
Mass of plant		The experiment is conducted with both plants; mustard and spinach, have an approximate value of mass at 5.0 g. Different mass of plants may lead to a highly different absorbance.	
Period of centrifuge		Both samples (Mustard and spinach), which in the form of extracted chlorophyll are both placed in the same time period in the centrifuge device.	
Volume of propanol		Fix the volume of propanol inserted into the mortar which is at $20cm^3$.	
Time of the test tube inserted into the dark phase		Fix the time period where the test tubes containing extracted chlorophyll of both plants to 20 minutes.	

Table 1: The table above shows the variables used in the experiment.

MATERIAL AND APPARATUS

Apparatus:

No.	Apparatus	Quantity	Volume/Size	Uncertainties
1.	Beaker	1	500 ml	Nil
2.	Tripod stand	1	20 ml	Nil
3.	Bunsen burner	2	1 meter	Nil
4.	Mortar and pestle	1	Standard size	Nil
5.	Measuring cylinder	3	100 ml	$\pm$0.1 ml
6.	Glass rod	1	Standard size	Nil
7.	Test tube	2	Standard size	Nil
8.	Centrifuge	1	Standard size	Nil
9.	Centrifuge tubes	4	100 ml	$\pm$0.05ml

10.	Conical flask	1	Standard size	Nil
11.	Set of calorimeter	1	Standard size	Nil
12.	Weight balance	1	Standard size	±0.001 g

Table 2: The table above shows the apparatus used during the conduct of the experiment

Material: (1ml = 1g of substance)

No.	Material	Quantity	Volume/Size	Uncertainties
1.	Fresh green leaves (Mustard and spinach)	1 basin	-	-
2.	Aluminium foil	1 basin	-	-
3.	Acetone	1 reagent bottle	20 ml	

Table 3: The table above shows the materials used during the experiment.

METHOD OF EXPERIMENT

1. Fresh leaves are dipped into boiling water.
2. The leaves are cut, placed in a mortar an grinded up.
3. 20 cm3 of propanone (acetone) are inserted and the mixture is grinded again to produce a concentrated chlorophyll extract.
4. The solution is put into the centrifuges for 10 minutes. The supernatant formed on top is put into the test tube with stopper and the test tube is wrapped with an aluminium foil.
5. The test tube is placed in the dark part of the lab for at least half an hour.
6. The cuvet is put into and examined by using a calorimeter.
7. The data are recorded.

DATA COLLECTION

Quantitative Data (Room temperature is 27°C)

Wavelength (nm, nanometer)	Absorption (%) (±0.01)			
	Standard Solution	Spinach sample		
		T1	T2	T3
440	0.00	2.00	2.00	2.00
470	0.00	1.90	1.88	1.96
490	0.00	0.97	0.84	1.08
520	0.00	0.76	0.64	0.73
550	0.00	0.77	0.50	0.15
580	0.00	1.03	0.76	0.87
590	0.00	1.16	1.19	1.13
680	0.00	1.35	1.44	1.39

Table 4: The table above shows the amount of absorption of wavelength by the spinach sample exposed to different wavelength, referred to standard solution and taken in 3 different trials.

Wavelength	Absorption (%) (±0.01)

(nm, nanometer)	Standard Solution	Mustard sample		
		T1	T2	T3
440	0.00	2.00	2.00	2.00
470	0.00	1.33	2.00	2.00
490	0.00	0.75	1.95	2.00
520	0.00	-0.30	1.44	1.87
550	0.00	0.58	1.55	1.88
580	0.00	-0.29	1.81	2.00
590	0.00	1.34	2.00	2.00
680	0.00	0.43	1.87	1.91

Table 5: The table above shows the amount of absorption of wavelength by the mustard sample exposed to different wavelength, referred to standard solution and taken in 3 different trials.

Qualitative Data

Observations
Both spinach and mustard are green in colour
Both spinach and mustard becomes softer after being dipped into the boiling water
The extraction of mustard and spinach produced green colour
Both samples of extraction of chlorophyll of mustard and spinach produce two layers after being rotated in the centrifuge. The supernatant is dark green in colour and the debris is light green in colour.

Table 6: The table above shows the observations during the experiment.

DATA PROCESSING

Quantitative Data

The average percentage of absorbance of wavelength (nm) for the spinach samples exposed in different wavelengths (With respect to standard solution at 0.00nm) in different trials is calculated by using the following formula below:

$$\frac{T1 + T2 + T3}{n}$$

Where,

T1: Percentage of Absorbance in trial 1 (nm)

T2: Percentage of Absorbance in trial 2 (nm)

T3:Percentage of Absorbance in trial 3 (nm)

N= Number of trials

E.g: The calculation of the average percentage of absorbance of wavelength (nm) for the spinach sample exposed at 440 nm (With respect to standard solution at 0.00nm) in different trials is given below:

= [2.00+2.00+2.00]/3

= 2.00

Hence,

Wavelength (nm)	Average percentage of absorbance of wavelength by spinach sample (With respect to standard solution at 0.00
440	2.00
470	1.91
490	0.96
520	0.71
550	0.47
580	1.00
590	1.16
680	1.39

Table 6: Average percentage of absorption by spinach sample (%)

E.g: The calculation of the average percentage of absorbance of wavelength (nm) for the mustard sample exposed at 440nm (With respect to standard solution at 0.00nm) in different trials is given below

= [2.00+2.00+2.00]/3

= 2.00

Hence,

Wavelength (nm)	Average percentage of absorbance of wavelength by mustard sample (With respect to standard solution at 0.00 absorbance)
440	2.00
470	1.80
490	1.60
520	1.00
550	1.34
580	1.17
590	1.78
680	1.40

Table 7: Average percentage of absorption by spinach sample (%)

Standard Deviation for the average percentage of absorbance of wavelengths by both mustard sample and spinach sample (With respect to standard solution at 0.00

The calculation for the standard deviation for the average percentage of absorbance of wavelengths by both spinach and mustard samples (With respect to standard solution at 0.00 absorbance) is calculated by using the following formula:

$$\text{Standard deviation} = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N-1}}$$

Where,

x: Percentage Absorbance of the samples (Spinach or mustard) exposed in different wavelengths

$\bar{x}$: Average percentage of absorbance of wavelengths by samples (Spinach or mustard) (with respect to standard solution at 0.00 absorbance)

N: Number of trial

E.g. The standard deviation for the average percentage of absorbance of wavelengths by spinach sample (With respect to standard solution at 0.00 absorbance) for the wavelength of 440 nm:

$$\text{Standard deviation at wavelength 440nm} = \sqrt{\frac{(2.00-2.00)^2+(2.00-2.00)^2+(2.00-2.00)^2}{3-1}}$$

$= \pm 0.00$ % absorbance

Wavelength (nm)	Average percentage absorbance of wavelength by spinach sample (With respect to standard solution at 0.00 absorbance)	Standard deviation of the average percentage absorbance of wavelength
440	2.00	$\pm$0.000
470	1.91	$\pm$0.042
490	0.96	$\pm$0.12
520	0.71	$\pm$0.062
550	0.47	$\pm$0.31
580	1.00	$\pm$0.20
590	1.16	$\pm$0.03
680	1.39	$\pm$0.045

Table 8: The table above shows the standard deviation of the average percentage of absorption of wavelength of spinach

E.g. The standard deviation for the average absorbance of wavelengths by mustard sample (With respect to standard solution at 0.00 absorbance) for the wavelength of 440 nm:

Standard deviation at wavelength 440nm

$$= \sqrt{\frac{(2.00-2.00)^2+(2.00-2.00)^2+(2.00-2.00)^2}{3-1}}$$

$= \pm 0.00$ % absorbance

Hence,

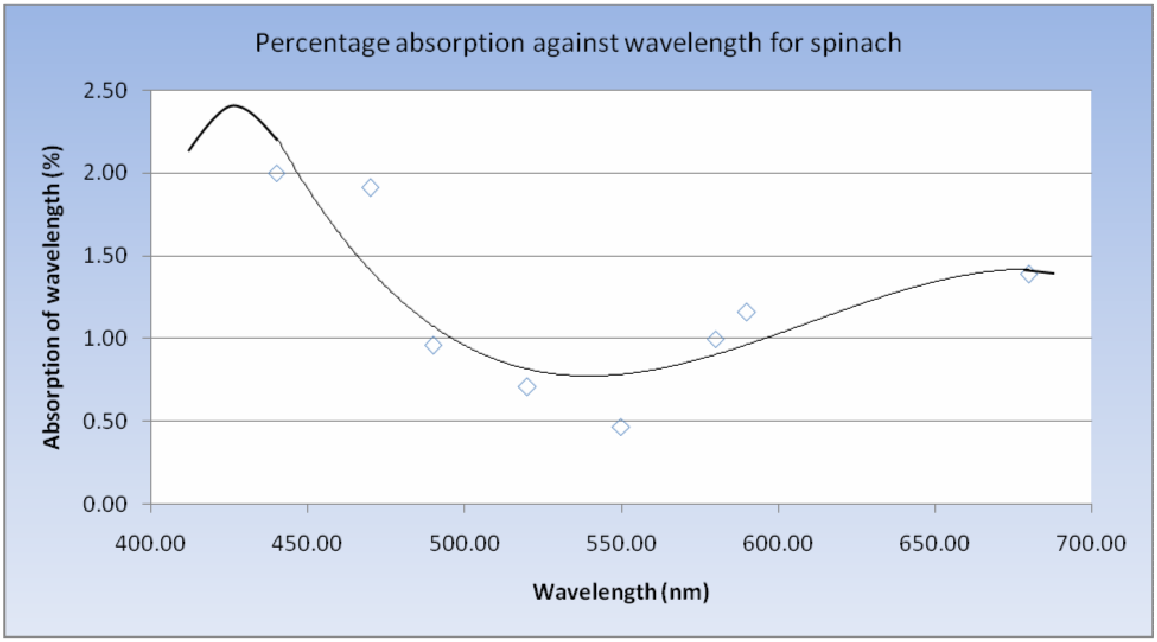
Wavelength (nm)	Average percentage absorbance of wavelength by mustard sample (With respect to standard solution at 0.00 absorbance)	Standard deviation of the average percentage absorbance of wavelength
440	2.00	$\pm$0.00
470	1.80	$\pm$0.40
490	1.60	$\pm$0.71
520	1.00	$\pm$1.15
550	1.34	$\pm$0.68
580	1.17	$\pm$1.27
590	1.78	$\pm$0.40
680	1.40	$\pm$0.84

Table 9: The table above shows the standard deviation of the average percentage of absorption of wavelength of mustard.

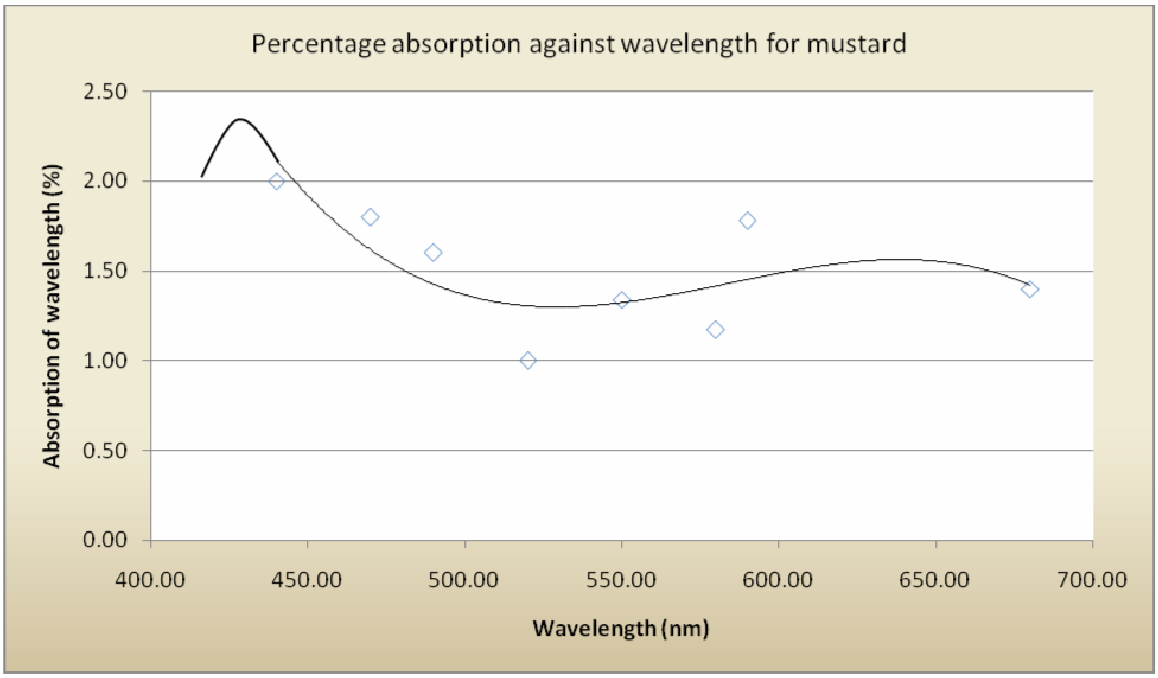
B) Qualitative Data

Observation	Explanation
Both plants green in colour	They contain photosynthetic pigment that reflects green colour.
Both plants become softer/wilt after being added with boiled water.	The high temperature of the water causes the membrane of the cell to burst off. No cell wall to keep the shape of the cell to be rigid.
Extraction of both plants produce green colour.	Since the cell membrane of the plant is absence after they have being boiled with boiled water, the chloroplast are removed easier. This photosynthetic pigment is the one that reflects green colour.
Two layers formed in the centrifuge tubes	Two layers formed. The supernatant is at the top as it ahs a lower density than that of the debris below the centrifuge tube.

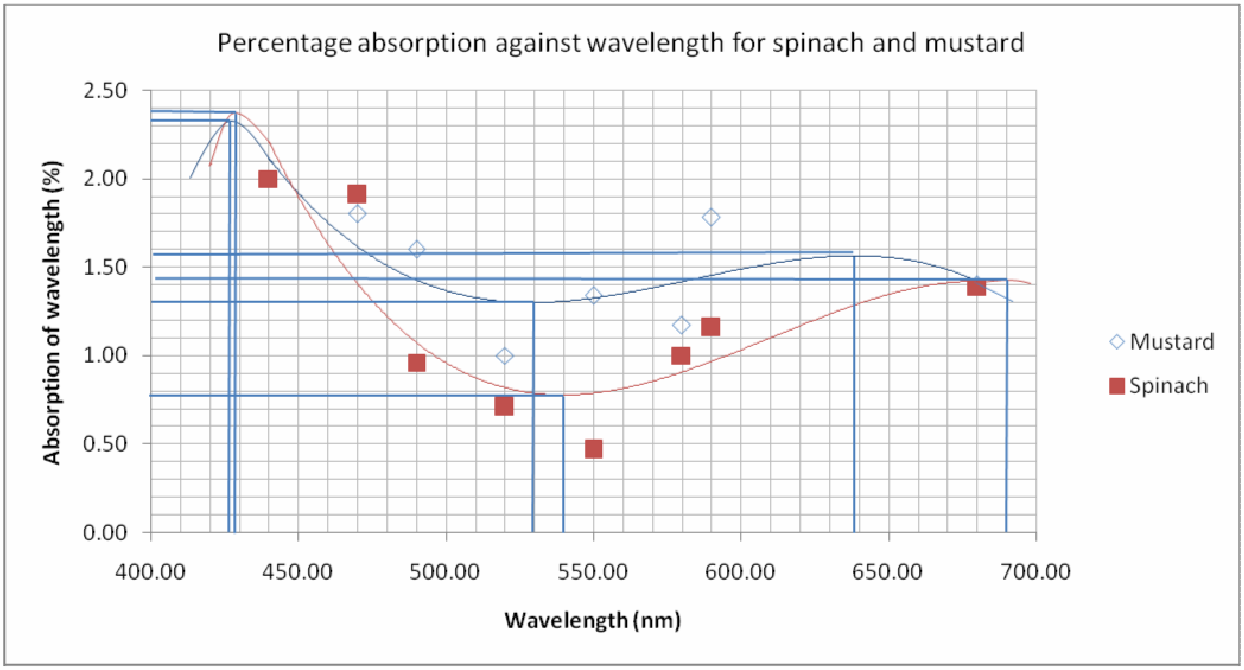
Table 10: The table above shows the explanation.



Graph 1: The percentage of absorption against wavelength of spinach sample



Graph 2: The percentage of absorption against wavelength of mustard sample



Graph 1 shows the graph of rate of photosynthesis od disk leaf against distance of the light source from the beaker.

DISCUSSION

a) The photosynthetic pigments are materials that are responsible for absorbing and trapping the light energy in the earlier steps of photosynthesis. One of the major pigments of photosynthetic materials is chlorophylls. Chlorophylls is a mixture of pigments that comprises of chlorophyll a, chlorophyll b and some carotenoids like B-carotene, Xantophyll and phaeophytin. However, other types of chlorophyll like chlorophyll c and bacteriochlorophyll are found in non green plant, protistans and photosyntehetic bacteria.

b) The main objective of this experiment is to prove the fact that different form of plant which contains different concentration/number of photosynthetic pigments is tend to absorb different value of wavelength, hence, its different colour. Earlier hypothesis suggests that the wavelength will be absorbed by the photosynthetic pigment at a different absorption. Different wavelength which is absorbed or reflected by the photosynthetic pigments will determined the colour observed on the leaves. For example, two samples that are used in the experiment is spinach and mustard plant. In the earlier observation, it can be seen that the spinach plant is greener than that of mustard plant which a little bit light green. Hence a hypothesis is formed that the photosynthetic pigments in the spinach plant is able to absorb less amount of green

wavelength compared to that of a mustard plant. Hence, more green wavelengths will be reflected by the pigments in spinach plant than that in mustard.

c) The experiment is conducted by having two different plants; spinach and mustard that shows a small difference in their green colors. The plant is dipped into the boiled water first in order to smoothen them up for the grinding process. The boiling water will break the membrane of the cell and cause the chlorophyll easier to be extracted. During the grinding process in the mortar, propanone liquid is added in order to separate the chlorophyll extract from the plant through the process of filtration and is inserted inside a centrifuge tube. The tube later on is inserted into the centrifuge tube and being rotated. The product is a form of chlorophyll extract in partially liquid form. It can be observed that the both supernatant and the debris of the chlorophyll extract are presence inside the tube. The supernatant is inserted inside a test tube and is left in the dark room for 20 minutes. This process is important to prevent the bleaching of chlorophyll.

d) The data collected and observed is processed. Based on graph 1, it can be seen that two peaks is formed. One is near to the wavelength value of 425 nm and another one is near to 650 nm. Another peak is also formed at the through of the graph which is at 540 nm. These two peaks indicate that there is a very high absorption of wavelength. The peak at 425 nm shows absorption of 2.40 % of absorption and another peak at 650 nm shows 1.50 % of absorption. Hence more wavelengths is absorbed than reflected by the photosynthetic pigments of spinach at point 425 nm and 650 nm. However, at the bottom of the graph, it can be deduced there is a low absorption of wavelengths at point of 540 nm which is at 0.75%. Thus more wavelengths are reflected rather than being absorbed at this point.

e) Another separate data is taken with mustard as its sample. Based on graph 2, like spinach, the graph also indicated the presence of two peaks. One is near to 425 nm and another one is around 680 nm. Another peak is formed at the bottom/through of the graph at point of wavelength of 530 nm. These two peaks indicate that the wavelengths at these two points are absorbed most by the photosynthetic materials of mustard. The wavelength of 425 nm is absorbed with a percentage of 2.40% and another peak with wavelength 680 shows percentage absorption of 1.40 %. Hence, more wavelengths is absorbed rather than reflected at these points of wavelengths. However, at the bottom of the graph, it can be deduce that the mustard sample does not really absorb much wavelengths at point 540 nm with an absorption percentage of 1.30%.

f) Graph 3 shows the combination of both percentage of absorption against wavelengths for both samples; spinach and mustard. Based on the diagram, it can be deduced that the peak for the top and bottom of the graphs are nearly the same for both samples.

However the point of difference will stand at the percentage of absorption of peak at the bottom of both graphs. For spinach, the minimum point of the percentage of absorption is at 0.75% while the minimum point for the mustard sample is at 1.30%.

g) The first peak indicates the percentage of absorption of wavelengths that produce blue colour. The second peak for both graphs indicates the percentage of absorption of wavelengths that produce red colour. The minimum point of the percentage of absorption for both graphs indicates the absorption of wavelengths that produce green colour. Hence, the absorption of green colour in the chlorophyll pigments in spinach is lower than that of mustard. This means that greener colour is reflected by the spinach compared to mustard. This explains a darker green colour of spinach than mustard.

LIMITATION AND EVALUATION

Limitations	Suggestions
The experiment is conducted with an uneven distribution of wavelength interval.	The wavelength interval imposed by the colorimeter is not even and does not have an equal range of wavelength. This can affect the value of the experiment as it is possible for a peak to exist between those large differences. Hence, it is suggested that a more proper device should be used with an equal, or can be determined on our own. A UV spectrophotometer can be used in which it fulfilled the whole requirements mentioned.
The experiment is exposed too much human and technical error as different groups will have more students handling one responsibility.	The presentation of the data is managed by a group of data collected and shared among students. The uncertainties that may exist is that when a group of students has produced data that is wrong, then it will affect the whole calculation and the presentation of the absorption percentage by the graphs.
The boiling water is not that boiled up	When the boiling water is added into the beaker containing both mustard and spinach, the boiling water is not boiled enough. This will make some of the leaves not to wilt properly and probably can affect the result of the data at the end of the experiment. The membrane will not be fully permeable and can make it harder for the chlorophyll to be extracted. The suggested solution is to add water that is just boiled.
The dark period is not even	The test tubes containing the chlorophyll extract are then taken into dark places for 20 minutes. However, different groups will assign their test tubes in different location in which the intensity of the darkness may be uneven. Hence this can effect the measurement by the calorimeter. The suggested solution is to gather all the test tubes into one place that has an even distribution of darkness intensity.
The uncertainty of the	Since digital stopwatch provides less uncertainty than that of

analog stopwatch is larger than that of digital stopwatch	analog stopwatch, it is better to use digital stopwatch for the time taken for the test tube inside the centrifuge, the time taken for the test tube to be left in the dark room. This will provide a better and more specific time interval to avoid large uncertainties.
The aluminium foil does not fully covered the test tube	The aluminium foil is used to cover the test tubes before they are left in the dark place. The aluminium foil is not wide enough to cover the whole test tube. However, once the test tube is not fully covered by the aluminium foil, there is a probability that some light can be passed through into the test tube. This will affect the data of absorption later on. Hence, a larger piece of aluminium foil is needed to cover the whole test tube.

The table above shows the limitation and suggestion of the experiment

CONCLUSION

Based on the result of the experiment, it can be deduced that the reason on why most plants produced green colors is because the presence of the photosynthetic pigments which absorb certain lights and reflect certain lights. The graph of the percentage of absorption clearly shows that the spinach and mustard absorb both blue and red colour with an exception of green colour. On the graph, it can be proven that the spinach will absorb less green wavelength than spinach; hence it's greener colour than mustard. The hypothesis is accepted.

APPENDIX

Quantitative Data

The average percentage of absorbance of wavelength (nm) for the spinach samples exposed in different wavelengths (With respect to standard solution at 0.00nm) in different trials is calculated by using the following formula below:

$$\frac{T1 + T2 + T3}{n}$$

= [2.00+2.00+2.00]/3

= 2.00

Hence,

Wavelength (nm)	Average percentage of absorbance of wavelength by spinach sample (With respect to standard solution at 0.00
440	[2.00+2.00+2.00]/3 = 2.00
470	[1.90+1.88+1.96]/3 = 1.91
490	[0.97+0.84+1.08]/3 = 0.96

520	[0.76+0.64+0.73]/3 = 0.71
550	[0.77+0.50+0.15]/3 = 0.47
580	[1.03+0.76+0.87]/3 = 1.00
590	[1.16+1.19+1.13]/3 = 1.16
680	[1.35+1.44+1.39]/3 = 1.39

Table 6: Average percentage of absorption by spinach sample (%)

Wavelength (nm, nanometer)	Absorption (%) (±0.01)			
	Standard Solution	Mustard sample		
		T1	T2	T3
440	0.00	2.00	2.00	2.00
470	0.00	1.33	2.00	2.00
490	0.00	0.75	1.95	2.00
520	0.00	-0.30	1.44	1.87
550	0.00	0.58	1.55	1.88
580	0.00	-0.29	1.81	2.00
590	0.00	1.34	2.00	2.00
680	0.00	0.43	1.87	1.91

Wavelength (nm)	Average percentage of absorbance of wavelength by mustard sample (With respect to standard solution at 0.00 absorbance)
440	[2.00+2.00+2.00]/3 = 2.00
470	[1.33+2.00+2.00]/3 = 1.80
490	[0.75+1.95+2.00]/3 = 1.60
520	[-0.30+1.44+1.87]/3 = 1.00
550	[0.58+1.55+1.88]/3 = 1.34
580	[-0.29+1.81+2.00]/3 = 1.17
590	[1.34+2.00+2.00]/3 = 1.78
680	[0.43+1.87+1.91]/3 = 1.40

Table 7: Average percentage of absorption by spinach sample (%)

The calculation for the standard deviation for the average percentage of absorbance of wavelengths by both spinach and mustard samples (With respect to standard solution at 0.00 absorbance) is calculated by using the following formula:

$$\text{Standard deviation} = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N-1}}$$

Where,

x: Percentage Absorbance of the samples (Spinach or mustard) exposed in different wavelengths

$\bar{x}$: Average percentage of absorbance of wavelengths by samples (Spinach or mustard) (with respect to standard solution at 0.00 absorbance)

N: Number of trial

Wavelength (nm)	Average percentage absorbance of wavelength by spinach sample (With respect to standard solution at 0.00 absorbance)	Standard deviation of the average percentage absorbance of wavelength
440	2.00	$\sqrt{\frac{(2.00-2.00)^2+(2.00-2.00)^2+(2.00-2.00)^2}{3-1}} = \pm 0.000$
470	1.91	$\sqrt{\frac{(1.90-1.91)^2+(1.88-1.91)^2+(1.96-1.91)^2}{3-1}} = \pm 0.042$
490	0.96	$\sqrt{\frac{(0.97-0.96)^2+(0.84-0.96)^2+(1.08-0.96)^2}{3-1}} = \pm 0.12$
520	0.71	$\sqrt{\frac{(0.76-0.71)^2+(0.64-0.71)^2+(0.73-0.71)^2}{3-1}} = \pm 0.062$
550	0.47	$\sqrt{\frac{(0.77-0.47)^2+(0.50-0.47)^2+(0.15-0.47)^2}{3-1}} = \pm 0.31$
580	1.00	$\sqrt{\frac{(1.03-0.89)^2+(0.76-0.89)^2+(0.87-0.89)^2}{3-1}} = \pm 0.20$
590	1.16	$\sqrt{\frac{(1.16-1.16)^2+(1.19-1.16)^2+(1.13-1.16)^2}{3-1}} = \pm 0.03$
680	1.39	$\sqrt{\frac{(1.35-1.39)^2+(1.44-1.39)^2+(1.39-1.39)^2}{3-1}} = \pm 0.045$

Table 8: The table above shows the standard deviation of the average percentage of absorption of wavelength of spinach

Wavelength (nm)	Average percentage absorbance of wavelength by mustard sample (With respect to standard solution at 0.00 absorbance)	Standard deviation of the average percentage absorbance of wavelength
440	2.00	$\sqrt{\frac{(2.00-2.00)^2+(2.00-2.00)^2+(2.00-2.00)^2}{3-1}} = \pm 0.00$
470	1.80	$\sqrt{\frac{(1.33-1.78)^2+(2.00-1.78)^2+(2.00-1.78)^2}{3-1}} = \pm 0.40$

490	1.60	$\sqrt{\frac{(0.75-1.57)^2+(1.95-1.57)^2+(2.00-1.57)^2}{3-1}} = \pm 0.71$
520	1.00	$\sqrt{\frac{(0.30-1.20)^2+(1.44-1.20)^2+(1.87-1.20)^2}{3-1}} = \pm 1.15$
550	1.34	$\sqrt{\frac{(0.58-1.34)^2+(1.55-1.34)^2+(1.88-1.34)^2}{3-1}} = \pm 0.68$
580	1.17	$\sqrt{\frac{(0.29-1.37)^2+(1.81-1.37)^2+(2.00-1.37)^2}{3-1}} = \pm 1.27$
590	1.78	$\sqrt{\frac{(1.34-1.78)^2+(2.00-1.78)^2+(2.00-1.78)^2}{3-1}} = \pm 0.40$
680	1.40	$\sqrt{\frac{(0.43-1.40)^2+(1.87-1.40)^2+(1.91-1.40)^2}{3-1}} = \pm 0.84$

Table 9: The table above shows the standard deviation of the average percentage of absorption of wavelength of mustard.

REFERENCE

1. Biology Oxford, Standard and Higher Level, Andrew Allot: Topic; Photosynthesis
2. Http://wikipedia_chlorophyll.com
3. Biology for IB Diploma, Hodder Education, CJ Clegg, page 288