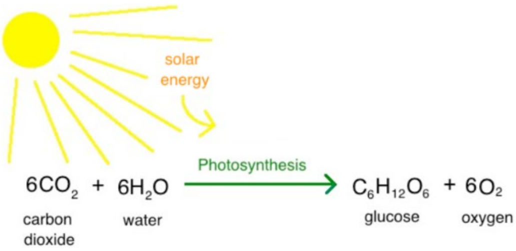
Chlorophyll Chromatography

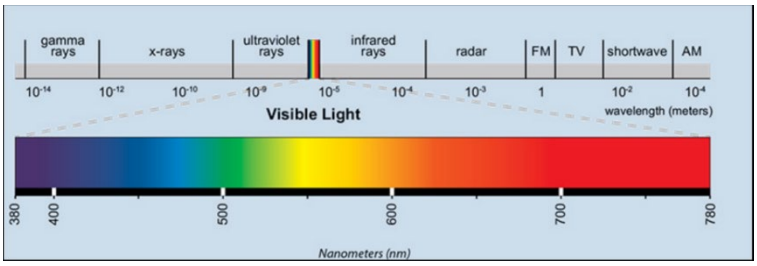
Aim:

To determine the best colour in the visible light spectrum for plant growth

Theory – light spectrum, photosynthesis, and plant pigments:

The light spectrum contains visible and invisible types of light. Plants absorb visible light for photosynthesis; this is the process by which plants use sunlight, carbon dioxide, and water to produce food in the form of glucose. Oxygen is also produced in this process; plants do not need oxygen, so it is released into the air for animals to breathe.





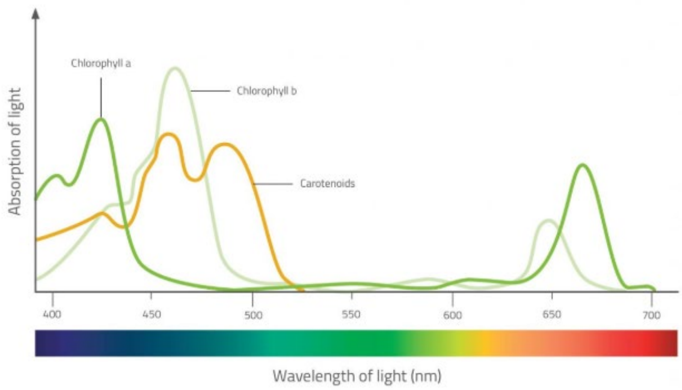
Each colour on the visible light spectrum has a different wavelength, and therefore a different amount of energy. The purple/violet end of the light spectrum has lots of energy and short wavelengths, however, red light has a lower amount of energy and longer wavelengths.

Each colour in the visible light spectrum is useful for plant growth and development:

- Blue light – encourages the growth of leaves
- Red light (combined with blue) – helps the plant to flower
- Green light – least effective for growth as plants do not absorb much green light – they reflect it (hence the green colour of leaves)

Leaves contain a variety of pigments. Each pigment absorbs a different wavelength of light. The main pigments are:

- Chlorophyll A
  – absorbs red and blue light
  – reflects green light
- Chlorophyll B
  – absorbs red and blue light
  – reflects green light
- Carotenoids
  – carotenes and xanthophylls
  - reflects yellow, orange, and red light
- Anthocyanins
- Flavonoids



Absorption spectrum for photosynthetic pigments

Chlorophylls are the main pigments needed for photosynthesis; however, carotenoids also help by absorbing more light. Anthocyanins and flavonoids are not required for photosynthesis, but instead determine the colour of any flowers on the plant. These colours help attract insects to pollinate the plant.

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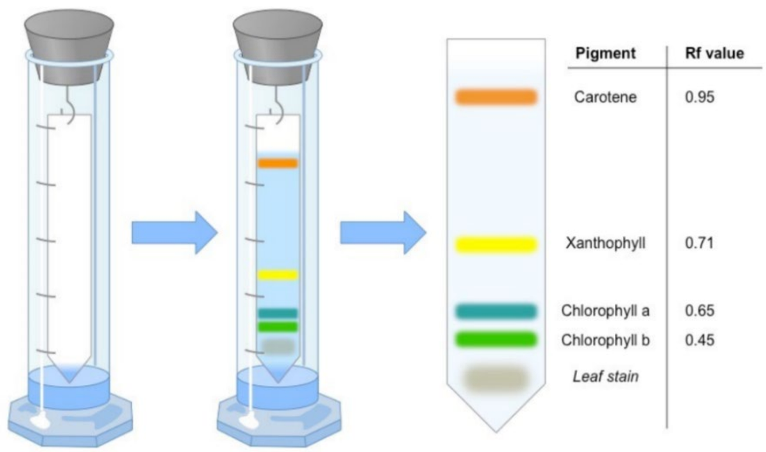
Theory – Chromatography:

Chromatography is a method used to separate mixtures. There are four main types of chromatography:

- Liquid Chromatography
- Gas Chromatography
- Thin-Layer Chromatography
- Paper Chromatography

Paper Chromatography is a useful method for analysing the types of pigments found in leaves. A strip of paper is placed in a solution of alcohol (such as methylated spirits) and leaf pigments. The solution is drawn up the paper due to capillary action, and the pigments are separated out as shown below:



Hypothesis:

Green light is the least effective colour in the visible light spectrum for plant growth, because it is not as useful to the plant and is reflected. Red and blue are more effective colours for plant growth and development, as they are used by the plant for photosynthesis.

Variables:

The independent variable

- The cellophane colour
  (this affects the colours in the visible light spectrum that will reach the plant).

The dependent variables:

- The height of the seedlings
- Any changes in plant growth or health (plant colour etc.)
- Changes in the pigments (measured using chromatography)

The controlled variables:

- The plants, seeds, and soil are the same for each sample
- The amount of water, light, and temperature
- The same containers/setup
- The same location

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Materials:

Planting and setting up:

- Three punnets of carrots
- Three punnets of peas
- 1 packet of broad beans
- 1 packet of alfalfa seeds
- Potting mix/soil
- 2 large boxes and containers for the plants
- Cellophane – clear, blue, red, green
- Cardboard
- Cello tape
- Ruler
- Scissors
- Pencil

Chromatography:

- Mortar and pestle
- Plastic cups
- Popsicle sticks (or similar)
- Clear Methylated Spirits
- Chromatography test paper

Method:

<u>Planting</u>

1. The potting mix was put in each of the four containers
2. On one side of each container, half a punnet of carrots and half a punnet of peas were planted – the two remaining punnets were put aside to be used for chromatography
3. On the other side of the containers, the broad bean and alfalfa seeds were planted
4. Each of the containers were lightly watered

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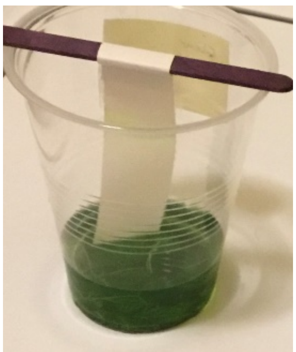
Setup of plant boxes

1. A piece of cardboard was cut to divide one box into two halves
2. Tape was used to secure the cardboard in place
3. One half of the box was covered with one sheet of coloured cellophane. The cellophane was secured in place with tape at the top, and clipped at the corners, so the plants could be removed
4. The other half of the box was covered with a different colour cellophane
5. This process was repeated for the other box using the remaining two colours
6. One container of plants was placed in each of the four sections
7. The boxes were placed on a table inside in full sunlight
8. Plant growth was measured at regular intervals, and the results were recorded in a spreadsheet for later analysis. Any observations regarding plant health were also recorded


Chromatography

The two remaining punnets of plants were used for the chromatography process.

1. The carrot leaves were cut off, placed in the mortar and ground up using the pestle.
2. The mixture was placed into a plastic cup and covered with clear methylated spirits.
3. The chromatography test paper was cut into strips approximately 2.5cm wide and taped to a popsicle stick
4. The stick and paper were balanced over the cup with the paper just touching the liquid
5. The solution was left for about two hours to allow the process of chromatography to occur
6. This process was repeated for the pea seedlings

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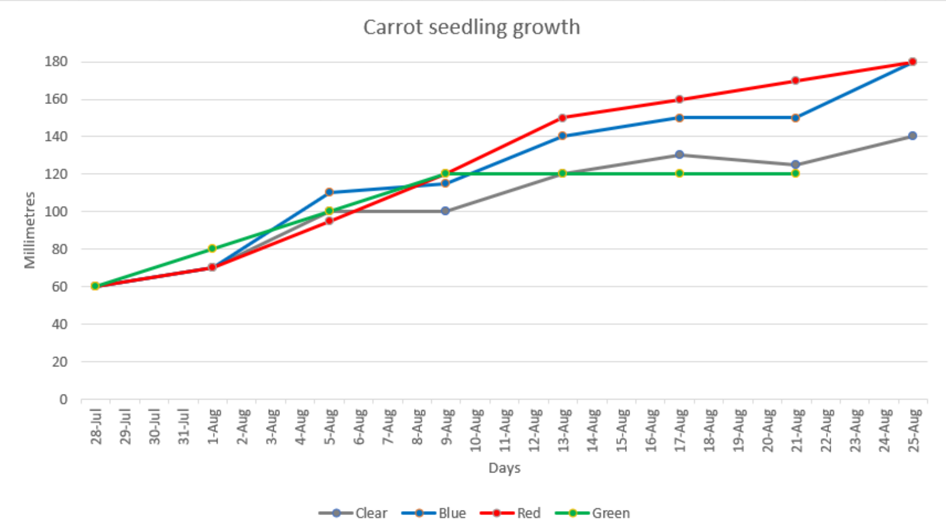
Results: Plant growth and development

The plant pots were examined every four days – the height of the plants was recorded, and observations were made regarding any major differences in the samples (such as colour of the leaves and stem strength). *No data was entered for plants that died during the experiment*

Carrots:

Carrot seedling growth in mm	28-Jul	1-Aug	5-Aug	9-Aug	13-Aug	17-Aug	21-Aug	25-Aug
Clear	60	70	100	100	120	130	125	140
Blue	60	70	110	115	140	150	150	180
Red	60	70	95	120	150	160	170	180
Green	60	80	100	120	120	120	120	



Observations:

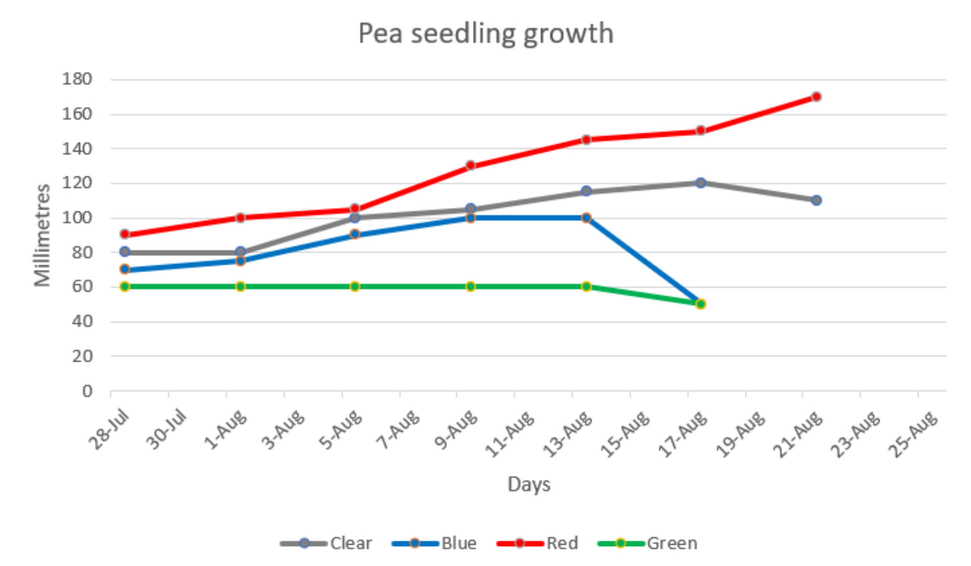
<u>13th August:</u>
The blue is very floppy, (and the red slightly) compared to the clear. The green looks a bit pale and some leaves have gone a little bit yellow.

<u>17th August:</u>
Red and blue are growing the tallest. The clear is the next best - the green is starting to go yellow and die off.

<u>25th August:</u>
The clear sample is floppy, turning yellow and a bit mouldy.
Blue and red are growing well but slightly yellow.
Green is shrivelled up and dead.

Peas:

Pea seedling growth in mm	28-Jul	1-Aug	5-Aug	9-Aug	13-Aug	17-Aug	21-Aug	25-Aug
Clear	80	80	100	105	115	120	110	
Blue	70	75	90	100	100	50		
Red	90	100	105	130	145	150	170	
Green	60	60	60	60	60	50		



Observations:

<u>13th August:</u>
The blue and green are breaking and starting to die.

<u>17th August:</u>
The red is the best but going a little bit yellow. The clear is starting to die. The blue and green have almost died.

<u>25th August:</u>
The clear is blackened and has died. The blue is mouldy and dead. The red has collapsed, and the green is very mouldy and dead.

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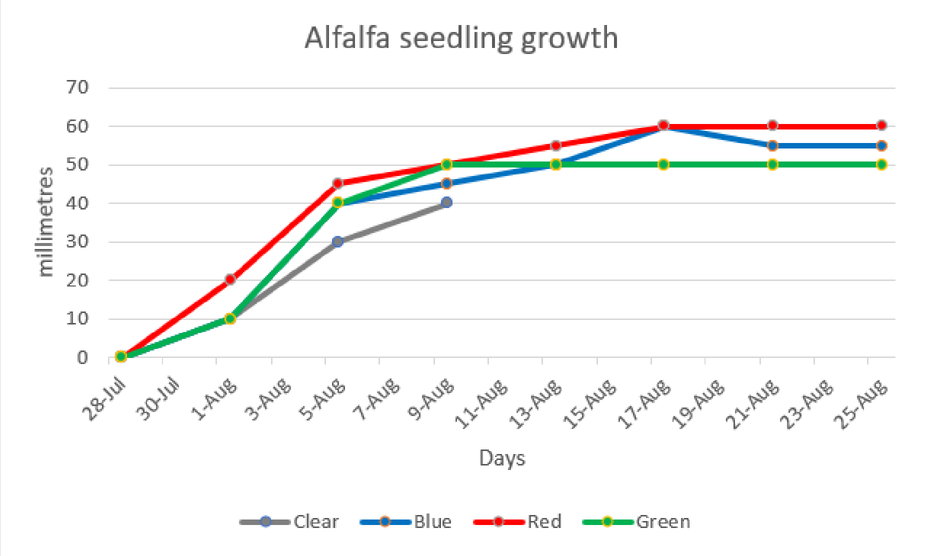
Alfalfa:

Alfalfa seedling growth in mm								
	28-Jul	1-Aug	5-Aug	9-Aug	13-Aug	17-Aug	21-Aug	25-Aug
Clear	0	10	30	40				
Blue	0	10	40	45	50	60	55	55
Red	0	20	45	50	55	60	60	60
Green	0	10	40	50	50	50	50	50



Observations:

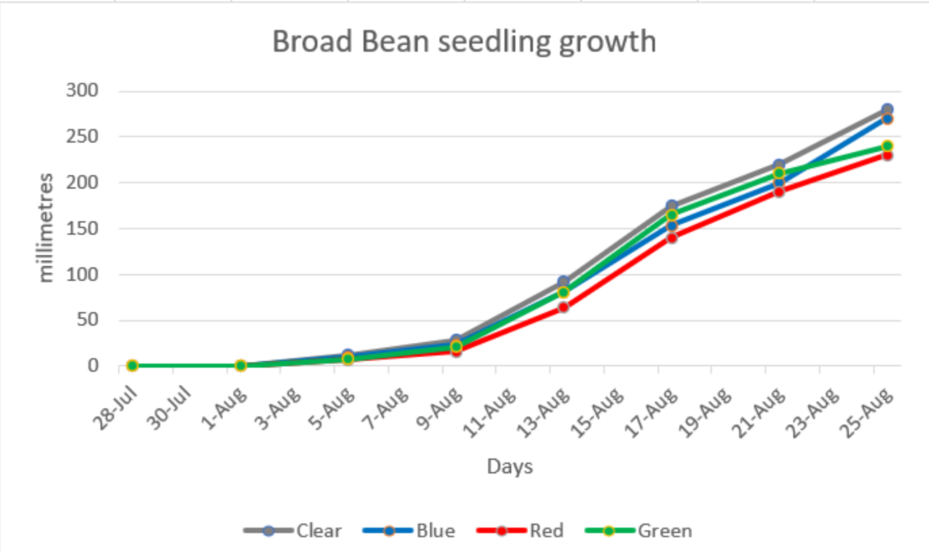
<u>13th August:</u>
Clear sample has died from mould. The green has mould starting. The red and blue samples look fairly healthy.

<u>17th August:</u>
Blue and red are growing well - just a little bit of yellow on them. The green is developing a bit of mould.

<u>25th August:</u>
The blue and red are starting to go yellow. The green is mouldy and going yellow.

Broad Beans:

Broad Bean seedling growth in mm								
	28-Jul	1-Aug	5-Aug	9-Aug	13-Aug	17-Aug	21-Aug	25-Aug
Clear	0	0	12	28	92	175	220	280
Blue	0	0	10	23	81	153	200	270
Red	0	0	7	16	64	140	190	230
Green	0	0	8	21	81	165	210	240
*averaged the height								



Observations:

<u>13th August:</u>
The clear and the blue are the best - lots of new leaves

<u>17th August:</u>
The clear and blue have the most leaves - healthy and in good condition. Red and green samples have fewer leaves and have black tips on the end of the leaves.

<u>25th August:</u>
They all have similar health, except the green has a few more black leaves

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PICTURES:

2nd August:

CLEAR **BLUE** **RED** **GREEN**

white marks on green sample leaves

9th August:

CLEAR **BLUE** **RED** **GREEN**

12th August:

CLEAR **BLUE** **RED** **GREEN**

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17th August:

CLEAR **BLUE** **RED** **GREEN**

25th August:

CLEAR **BLUE** **RED** **GREEN**

Green sample

Carrots seedlings have wilted and turned yellow.

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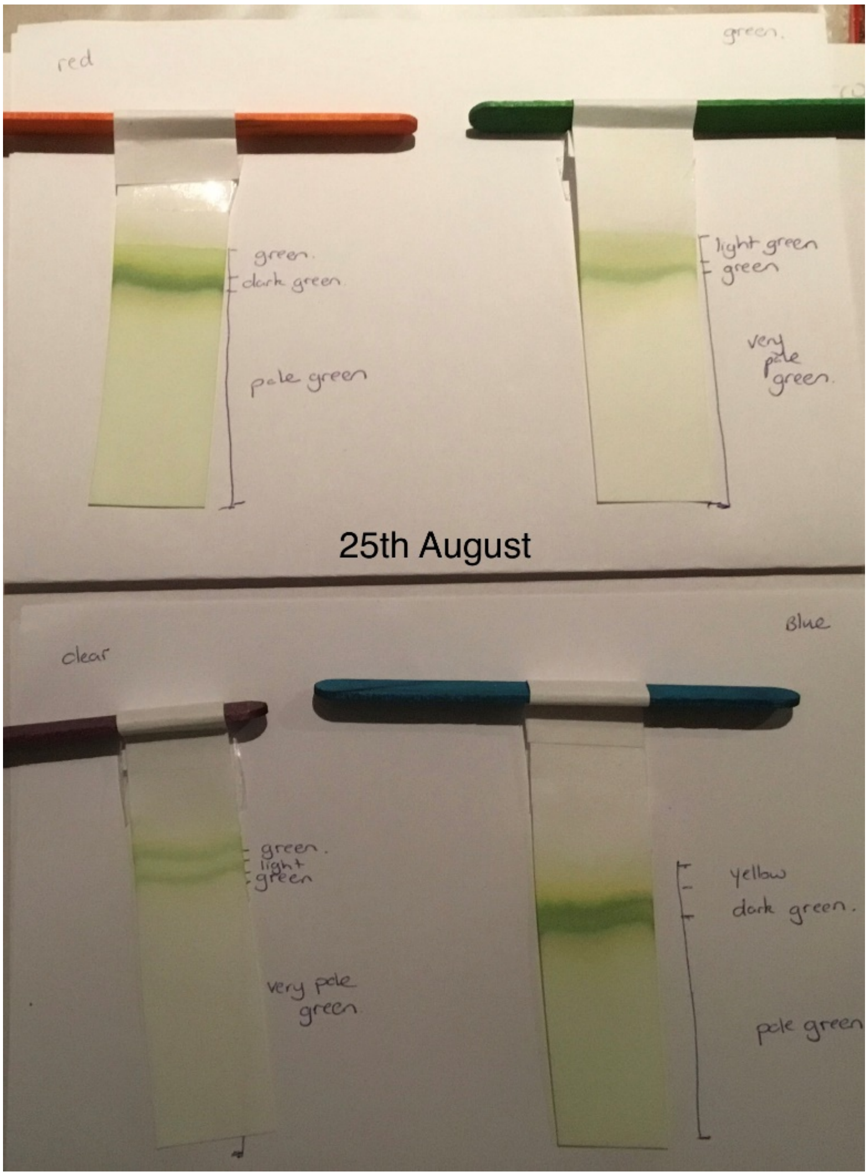
Results: Chromatography

Original test – peas and carrots:

End of experiment test – carrots only (peas had died):



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Discussion:

The data from the carrot seedling growth indicated that red and blue were the best colours for growth. Both seedling samples started at a height of 60mm and ended at 180mm; however, the stems weren't as strong as the seedlings in the clear sample. The next best colour was clear, as the plants grew to a height of 140mm. Green was clearly the worst colour, as the seedlings did not grow any more than 120mm high; it was the first sample to turn yellow and had shrivelled up and died by the end of the experiment. This supports the theory that green light is the least effective colour in the visible light spectrum for plant growth; red and blue are more useful colours for plants to grow.

The data from the pea seedlings showed that red was the best colour for plant growth. These seedlings almost doubled in height, growing from 90mm to 170mm. The clear and blue samples were similar; the seedlings increased in height by 30-40mm, before they started to die. The green seedlings did not change, as they started at a height of 60mm and had no further growth. By the end of the experiment, all the pea seedlings were dead; the green and blue samples grew mould and died the quickest. This again supports the theory that green light does not promote plant growth, whilst the red showed strong growth. The blue was growing better than the green, however, it grew mould and died.

The alfalfa seed results showed that the red colour had slightly better growth, with the plants reaching a maximum height of 60mm. The blue seedlings grew almost as well, reaching 55mm; then the green sample, which grew to 50mm. The clear sample grew mould very quickly and died by day 12 (9^{th} August). The green also started to grow some mould by the end of the experiment, and all samples began to show yellowing leaves. Red and blue light are again proved to be better for plant growth than green. It was surprising that the clear sample did not grow well; this was probably due to the mould that developed.

All the broad bean samples grew well, however, the clear had the highest growth, increasing to 280mm on average. The clear and blue had the healthiest growth, with lots of new leaves; the red had slightly less growth than the other samples, reaching an average of 230mm. The red and green samples did not have as many leaves; there were also lots of black leaves in the green sample. Again, the green sample showed less healthy plants, and not as much growth compared to the blue sample. This was the only plant that grew slightly better with green than red.

At the beginning of the experiment, pea and carrot seedlings were each tested using chromatography. The results showed the presence of Chlorophyll B (yellow-green), Chlorophyll A (blue-green) and Xanthophyll (yellow).

After the experiment ended, each of the carrot samples were tested using chromatography to determine any change in leaf pigments. The red sample was similar to the original test, but showed a wider band of Chlorophyll A, and less Xanthophyll. The blue showed a much wider Chlorophyll A band to the original. The clear sample was lighter in colour, with no Chlorophyll A; the green sample was a lot lighter than the original, as it definitely had less green and yellow colours on the paper. This shows that there were definitely some pigment changes, due to the different coloured light, as all other factors were the same. The pea samples died, so they could not be tested again and compared to the original.

If this experiment were repeated, some improvements I would make include:

- I would conduct it during summer, so the plants could be outside in full sunlight – this would reduce the chance of mould
- I would use a wider variety of plants in larger pots, including some flowering plants
- I would learn more about chromatography and how to calculate Rf values
- I would test more plants at the beginning and end of the experiment to compare the Rf values – this would give me more accurate information about any pigment changes

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Conclusion:

My investigation proved that my hypothesis was correct; green light is less effective for plant growth than red and blue colours. In almost all of the samples, the plants grew best with red and blue light, and worse with green light.

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