Year 9 Research Project:

A Study on the Effects of Nitrogen Fertiliser versus Rhizobia Bacteria Inoculation on the growth of the Fava Bean plant

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Title

A study on the effects of nitrogen fertiliser versus rhizobia bacteria inoculation on the growth of the fava bean plant.

Abstract

This investigation compares the effect of added nitrogen fertiliser and rhizobia bacteria inoculation on the growth of the fava bean plant. Three groups of fava beans were planted, one group received nitrogen fertiliser, one group was inoculated with rhizobia bacteria and the third group was a control with no added nitrogen or rhizobia bacteria. Nitrogen fertiliser was added to the 'nitrogen fertiliser' group at the time of planting and then every two weeks. All pots received the same amount of light, water, temperature and humidity. Plant height was measured daily for six weeks and the wet and dry weights were measured at the end of the experiment. The results show that the plants inoculated with rhizobia bacteria were taller had greater biomass than the plants given nitrogen fertiliser and the control plants. The plants given nitrogen fertiliser were not as tall as the control plants but they had greater biomass.

Introduction

Nitrogen is an essential nutrient for plant growth, development and reproduction. Although nitrogen is one of the most abundant elements on earth, making up 78% of the air we breathe, nitrogen deficiency is one of the most common nutritional problems affecting plants worldwide. Plants require nitrogen because it is a major component of chlorophyll, which is essential for the process of photosynthesis. Nitrogen is also a major component of amino acids, required to make proteins, which are needed for the structural elements in the plant. Nitrogen is also essential for enzymes used in biochemical reactions and in the structure of DNA needed for growth and reproduction of the plants.

Plants obtain nitrogen through a number of sources. Bacteria and fungi in the soil break down organic matter from decaying plants and animals to provide organic nitrogen. Plants cannot use atmospheric nitrogen; it must be converted to other forms such as nitrates and ammonium to be utilised by the plant. Other free-living bacteria in the soil, known as nitrogen fixing bacteria, are able to extract nitrogen from the air and fix, or convert this nitrogen into ammonium or nitrate, which the plants are then able to utilise.

Rhizobia are nitrogen-fixing bacteria from the genus *Rhizobium*, these bacteria occur naturally in the soil. They are unique in that they are the only nitrogen-fixing bacteria that can form a symbiotic relationship with legumes, which are plants that bear seeds in pods. Unlike other soil bacteria, rhizobia cannot fix nitrogen alone, they need the legume plant. Rhizobia are very host-specific, that is only one species of rhizobia will only live with a particular type of legume. When soil nitrogen is low, legumes release a chemical known as flavonoids from their root hairs, which sends a signal to rhizobia indicating that the legume is seeking a symbiotic relationship. The bacteria then enter the root and release nodulation factor, which induces the formation of nodules on the roots of their host plants. Inside these nodules, the bacteria are able to convert nitrogen in the air into ammonium using the enzyme nitrogenase, which is supplied by the plant. This efficient use of nitrogen supplies the plants all its nitrogen needs without any wastage. Nitrogen fixation requires a lot of energy so the plant provides the bacteria with carbohydrates in the form of sugars.

Fertilisers added to the soil are another way in which plants can obtain sufficient nitrogen. This is important in agriculture to maximise growth and the yield of crops especially in areas where soil nitrogen and other nutrients levels are low. Fertilisers contain nitrogen in the form of ammonium; it also provides other nutrients essential for plant growth. Fertilisers are essential all over the world to grow crops however, the synthetic fertilisers are costly to make and require the burning of fossil fuels to produce, which can lead to atmospheric pollution. Nitrogen fertilisers are a major source of water pollution. If too little fertiliser is used, this can lead to insufficient growth, if too much fertiliser this can also be harmful to plants by drawing water out of the roots and stunting growth.

Fava beans, also known as broad beans, are legumes. They are a cold weather plant, ideal to grow in winter. These plants can form a symbiotic relationship with specific bacteria known as *Rhizobium leguminosarum*. This plant can develop nodules if planted in soil with existing rhizobia bacteria, otherwise inoculating (coating the seed with fresh bacteria) can help form a symbiotic relationship. With good nodulation, the bacteria can fix 90% of the nitrogen needs of the fava bean plant with little or no added fertiliser, which makes it a very efficient system. This bacterium has been shown to improve growth, speed up germination and help in the resistance of disease and stress in this plant.

Farmers have known for over 100 years that legumes yield good crops and have helped other crops grow by increasing soil nitrogen reserves and soil fertility. Studies have also shown that rhizobia may inhibit certain fungi in the soil and help speed up germination of seeds and this yields higher biomass, which is the dry mass of the plant. Once you have nitrogen fixing bacteria in the soil it may be present for future crops. Nitrogen fixating bacteria are the most cost efficient and environmentally friendly way to supply nitrogen to your plants.

This investigation aims to compare the growth of the fava bean plant inoculated with *Rhizobium leguminosarum* bacteria compared to the growth with nitrogen fertiliser. As nitrogen uptake translates into growth, the heights will be measured daily as well as the wet and dry weights at the end of the experiment.

Hypothesis

That the fava bean plants inoculated with rhizobia bacteria will grow taller and have greater biomass than the fava bean plants given nitrogen fertiliser.

Aim

To investigate whether nitrogen fertiliser or nitrogen fixation by rhizobia inoculation produces better growth of the fava bean plant as measured by height and biomass.

Materials

- 9 identical plastic plant pots with drainage holes.
- 9 identical plastic pot trays
- 27 fava beans all approximately the same size (approx. 20mm length)
- 6 litre bag of 'Horitco' organic potting soil with no added fertiliser
- Rhizobia inoculant (1 packet) from The Green Harvest Company
- 3 small zip lock bags
- 'Thirve' nitrogen fertiliser
- Teaspoon
- 1 litre measuring cup
- Rulers in millimetres
- Wooden skewers
- Measuring tape in millimetres
- Kitchen scale (grams with 2 decimal places)
- Tap water
- 20 ml syringe
- White labels
- Black marker
- Camera
- Magnifying glass
- Kitchen oven
- Metal baking trays

Materials used in the experiment

Method

1. 2 cups of potting soil were added to 9 identical plastic pots with drainage holes.

2. The 9 plastic pots were placed on 9 identical plastic trays

3. Using a black marker and white labels, 3 pots were labelled ‘no nitrogen added’, 3 pots were labelled with ‘nitrogen fertiliser’ and 3 pots were labelled with ‘Rhizobia bacteria.’

4. 3 separate zip lock bags were labelled 1, 2 and 3. 9 fava bean seeds were added to each with 1 teaspoon of tap water.

5. Half a teaspoon of the rhizobia inoculant was added to zip lock bag 1 and shaken until the seeds were coated with the inoculant. (This was done according to the instructions from the company that supplied the inoculant and fava beans)

6. 9 fava beans with rhizobia inoculant from zip lock bag 1 were divided equally into each of the 3 pots labelled ‘Rhizobia bacteria’ The seeds were evenly spaced and placed approximately 6 cm deep then covered with soil, making a total of 3 seeds in each pot.

7. 9 fava beans seeds from zip lock bag 2 were divided equally into each of the 3 pots labelled ‘nitrogen fertiliser.’ The seeds were evenly spaced and placed approximately 6 cm deep then covered with soil, making a total of 3 seeds in each pot.

8. 9 fava bean seeds from zip lock bag 3 were divided into each of the 3 pots labelled ‘no nitrogen fertiliser’. The seeds were evenly spaced and placed approximately 6 cm deep then covered with soil, making a total of 3 seeds in each pot.

9. 15 ml of tap water was added to the pot labelled ‘no nitrogen fertiliser’ and ‘Rhizobia bacteria’. 15 ml of nitrogen fertiliser was added to the pot labelled ‘nitrogen fertiliser’

10. All 9 pots were placed next to a window where they all received the same amount of sunlight, humidity temperature.

11. The soil was checked daily, if it was dry, tap water was added in the same amounts to all pots to make it moist.

12. Nitrogen fertiliser was added every 2 weeks to the ‘nitrogen fertiliser’ pots as stated on the instructions on the fertiliser box. Since the nitrogen fertiliser was dissolved in tap water, water in the same amount was also added to the ‘no nitrogen’ and the ‘rhizobia bacteria’ plants in order to treat them the same way.

13. Photos, observations and height measurements in millimetres were taken daily at approximately the same time. A different ruler was used to measure each plant so that contamination of the pots with the rhizobia bacteria would not occur.

14. When plants started bending over, wooden skewers were added to support the plant to stop the stem from snapping.

15. At the end of the experiment the wet and dry weights of the plants were taken in grams with 2 decimal places. To measure wet weights, the plants were carefully removed from the pots and all excess soil was carefully brushed off and then weighed on a scale in grams and recorded. To get dry weights, the plants were placed on a metal baking tray and put in an oven at 60 degrees Celsius for 24 hours and then weighed.

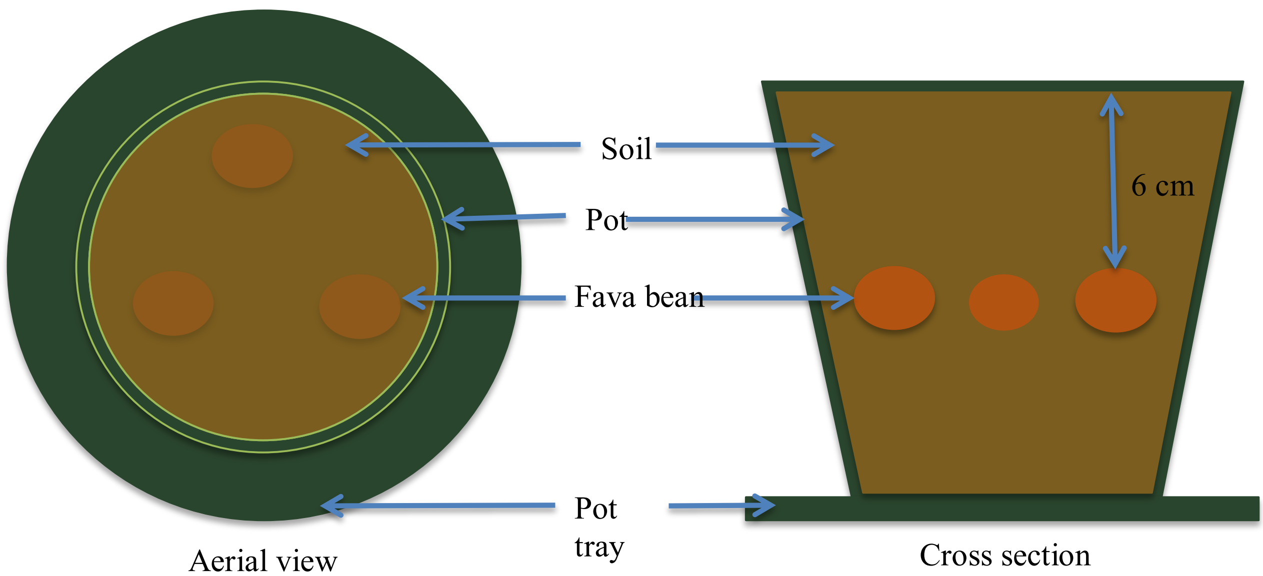
16. The percentage dry weight was calculated as dry mass/wet mass x 100.

Experiment Setup:

Control Plants

Nitrogen fertiliser

Rhizobia Bacteria



Variables

Independent variable

The presence of nitrogen fertiliser or rhizobia inoculant in the pots containing the fava beans.

Dependent variable

The height (measured in mm) and the wet and dry weights (grams) of the fava bean plants.

Controlled variables

Type and brand of fava bean seed
Size of fava bean seed
Light
Temperature
Humidity
Type and amount of soil
Type and size of plant pots and trays
Volume of tap water

Control experiment

Fava bean seeds with no added nitrogen fertiliser or Rhizobia bacteria.

Risk assessment

Risk	Danger	How risk will be addressed
Rhizobia bacteria	• Contamination of food products • Can make you sick	• Wear gloves • Wash hands after handling the rhizobia
Soil	• Contains live bacteria, viruses and protozoa • These organisms could contaminate food and make you sick • Can cause irritation to skin and eyes	• Wear gloves • Wash hands after use • Keep soil away from food preparation areas
Nitrogen Fertilizer	• Can cause skin and eye irritation • Can be toxic if ingested by children or pets	• Wear gloves • Wash hands after use • Keep out of reach of small children and pets
Fava beans	• Toxic if ingested by children or pets	• Keeps out of reach of small children and pets
Hot Oven	• Burning skin	• Wear oven mitts • Get parents to help

Results

Table 1: Average Height of Fava Bean plants (mm)

Day	Control	Nitrogen Fertilizer	Rhizobia
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	2.44	0	11.56
11	3.89	0	27
12	10.56	2.56	35.78
13	22.78	8.78	47.89
14	35.33	24.67	61.89
15	49.33	33.89	82.38
16	63.44	43.56	99
17	77.78	53.89	111.25
18	91.33	66.89	120.25
19	98.22	76.22	128.75
20	107.33	82.67	136.75
21	111	87.11	140.50
22	113.55	93.22	144.0
23	116.78	96.33	147.0
24	120.33	102.11	148.88
25	123.44	103.89	149.13
26	125.33	107.44	152

27	131.33	113.33	153
28	133	117.56	137
29	136.11	123.22	158.50
30	140.67	128.56	159.88
31	143.89	132.56	162.50
32	148.67	135.22	164.13
33	154.78	140.89	166.13
34	159.67	144.22	172.25
35	AWAY	AWAY	AWAY
36	AWAY	AWAY	AWAY
37	AWAY	AWAY	AWAY
38	191.11	177.11	203
39	200.67	186.78	210.5
40	205.11	191.89	218.13
41	209	197.67	225
42	212.22	200.78	229.38
43	213.89	203.56	231.5
44	219.56	207	236.13
45	222.44	210.11	240.63
46	223.67	212.22	242.63
47	228.44	205.44	248

Figure 1:

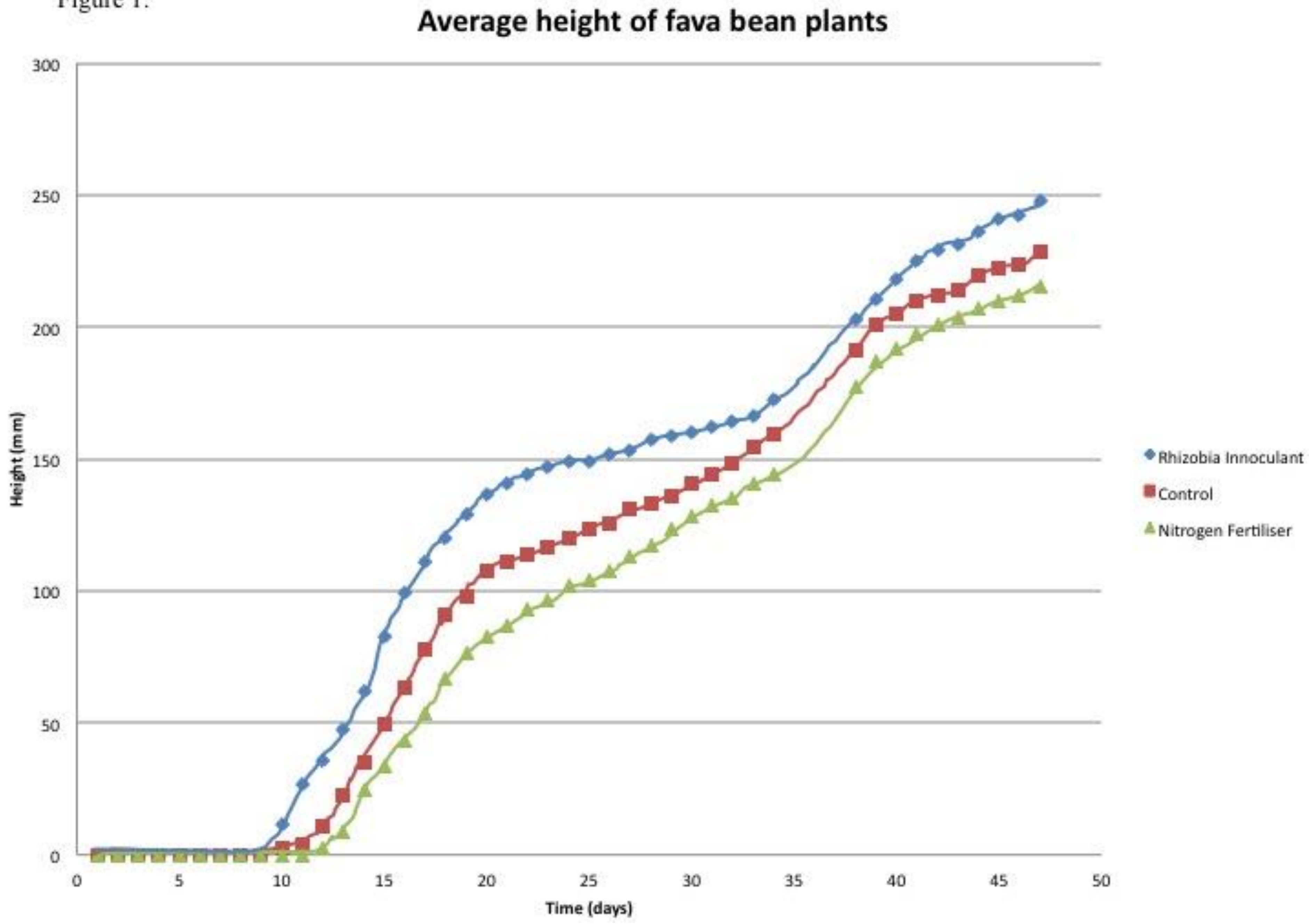

Average height of fava bean plants
Height (mm)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
50
Time (days)
Rhizobia Innoculant
Control
Nitrogen Fertiliser

Figure 2:

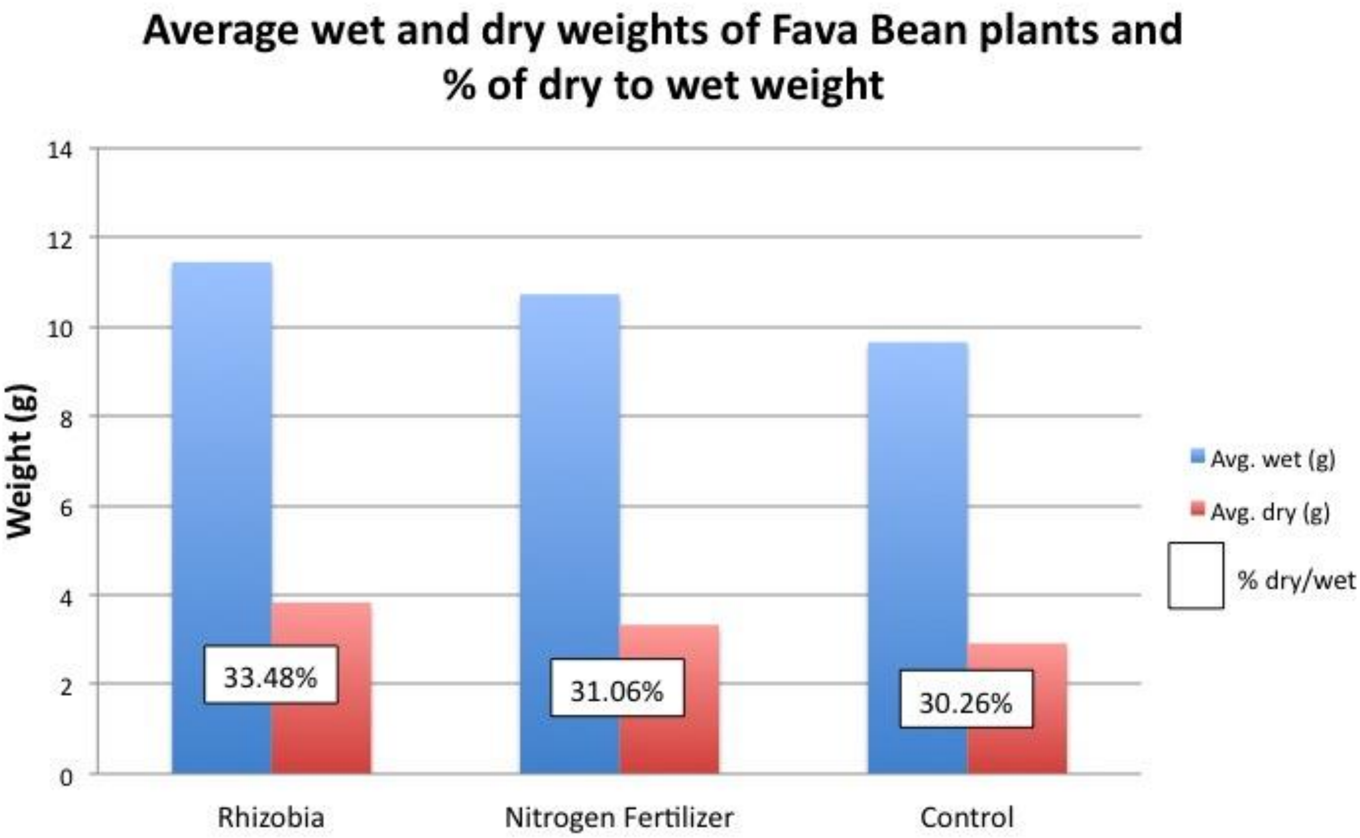
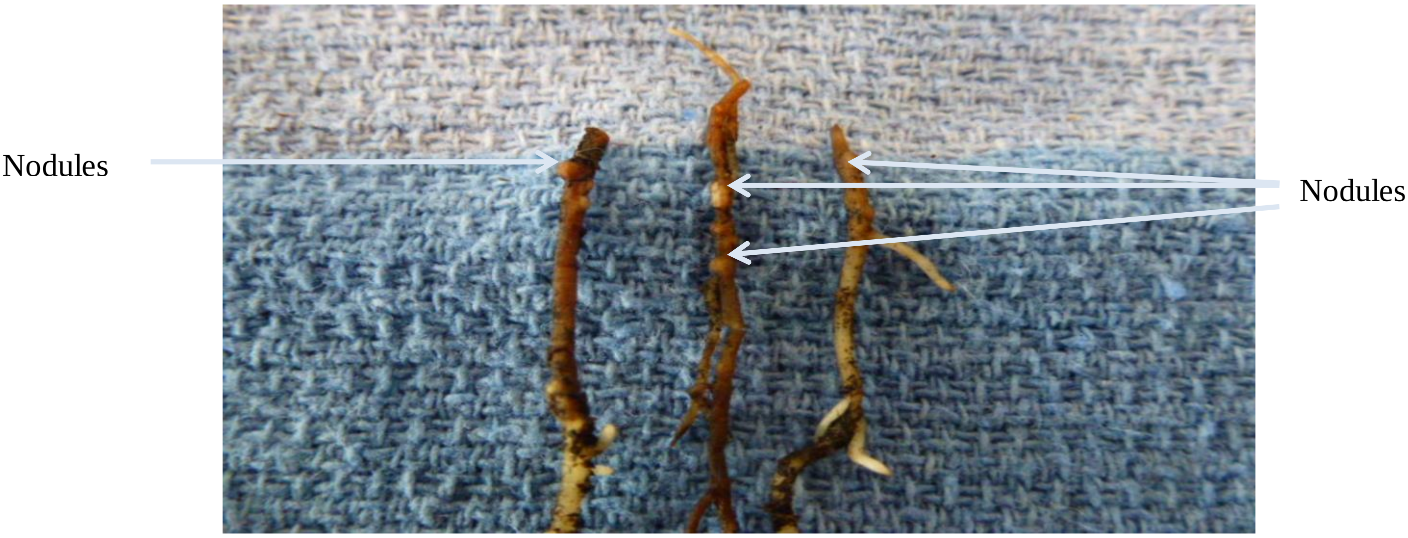


Table 2:

Observations during the growth of the fava bean plants

Rhizobia	Nitrogen Fertiliser	Control
Emerged first from the soil- Day 9: 50% seeds emerged Taller than nitrogen fertiliser and control plants Thicker roots than nitrogen fertiliser and control plants Roots had small nodules	Emerged last from the soil- Day 10: 22% seeds emerged Shortest group of plants out of the three groups. Some leaves have brown and burnt looking edges Leaves were slightly larger and more abundant than rhizobia and control plants Very thin roots observed	Emerged second from the soil- Day 9: 12.5% seeds emerged Taller than the nitrogen fertiliser plants but shorted than the rhizobia plants Very thin roots observed

Figure 3: Roots of Fava Bean plants after 47 days



Roots from rhizobia inoculated plants

Roots from nitrogen fertiliser plants

Roots from control plants

Figure 4: Fava bean plants after 47 days

Rhizobia inoculated

Nitrogen fertiliser

Control

Discussion

The results of this experiment reveal that after 47 days, the plants with rhizobia inoculant were taller on average and had greater average biomass than the plants with nitrogen fertiliser and the control plants. Table 1 shows that the average plant height for the rhizobia inoculant group was higher at 248 mm compared to the nitrogen fertiliser group at 205.44 mm and the control group at 228.44 mm. Figure 1 reveals that the rhizobia inoculated plants were consistently taller than the nitrogen fertilised group and the control group.

The rhizobium inoculant plants were the tallest, which is what was expected from the research. Inoculation of the fava bean seeds with rhizobia has been shown to speed up germination, promoting plant growth, nodulation and yield. Faster germination may be due to nod factor, which is produced by the bacteria, and can enhance germination of the seed. Also it has been found that rhizobium bacteria may inhibit growth of pathogenic fungi in the soil and therefore enhance germination. It was observed that the plants with the rhizobia inoculant were first to emerge from the soil compared to the nitrogen fertiliser group and the control group, this is probably due to faster germination in these pots leading to taller plants.

As well as speeding up germination, rhizobium inoculation allows the plant to fix nitrogen efficiently to make proteins which promote root, stem and leaf growth. Nitrogen fixation is very efficient in satisfying the high nitrogen requirements of legumes because the conversion of nitrogen to ammonium takes place inside the plant, this means all of the fixed nitrogen is readily available and in the form required to produce amino acids and proteins. By contrast, fertilisation can be a less efficient way of providing nitrogen to legumes. The added fertiliser may not be in sufficient amounts, or it may be excessive which can stunt root and plant growth. Fertilisers can be lost in the soil or get washed away and not get to the roots, therefore the plant must expend a lot of energy to move the nitrogen through the cell membranes from the soil to the roots. Fertiliser efficiency for legumes is generally 20-50% while nitrogen fixation is usually 90% efficient depending on soil and temperature conditions.

In this experiment, when the seeds were inoculated, a high concentration of bacteria coats the seeds so that as soon as the seed cracks, the bacteria can immediately enter the root and multiply to start forming nodules and start fixing nitrogen. Prior to nodulation, which takes about 1-2 weeks, the roots utilised nitrogen present in the soil. Once nodules are formed, the plant no longer takes nitrogen from the soil, the nitrogen needs can be achieved from bacterial fixation. The fact that the inoculated, seeds emerged faster from the soil means that they germinated faster and were able to absorb nitrogen sooner from the soil to start growing and could also start forming nodules for efficient nitrogen fixation. Faster germination lead to taller plants compared to the plants with added nitrogen and the control plants.

Figure 3 shows that inoculation was successful from the evidence of nodules on the roots of the plants inoculated with the rhizobium bacteria. Nodules were not obvious at first when the plants were taken out of the pots because there was so much soil covering the roots but on closer inspection after washing the roots in water and using a magnifying glass, nodules on the roots could be observed. These nodules were not present on the roots of the plants given nitrogen fertiliser or the control. The nodules were small approximately 1mm in size, pinkish in colour and located close to the crown of the roots, just as described in the articles in further research. Nodules are usually seen 5 weeks after planting the seeds and my plants were only 6 weeks old, if the experiment had been carried out for a longer period, perhaps the nodules may have been larger and more obvious. Even though nodules were evident, it is unclear whether they were poorly developed or healthy nodules since all the nodules observed in research papers were larger from mature plants about 100 days old where as the plants in this experiment were only 47 days old.

The plants given the nitrogen fertiliser were shorter than the control plants, which was an unexpected result. It was expected that the plants receiving nitrogen fertiliser would be taller than the control since they were getting more nitrogen. This result may be due to the fact that the plants given the nitrogen fertiliser may have received too much nitrogen, as the soil contained nitrogen from organic matter and the plants were given added nitrogen in the fertiliser. Although the instructions on the fertiliser packet were followed, the concentration made was for plants over a 4 square metre area, where as the pots used may have been too small for this concentration. Research has shown that excess nitrogen can lead to poor growth of the plant. Nitrogen increases chlorophyll production; this is done by creating larger leaf structures to allow for a bigger surface area for the photosynthesizing pigment. Having too much nitrogen can cause fast and excess foliage growth since energy originally intended for the growth of reproductive organs and root growth may be redirected towards leaf growth, which is likely to be a result of this. It was observed that the leaves on the plants with nitrogen fertiliser were larger and more in number than in the rhizobia inoculant group or the control. Also, fertilizer can increase the mineral salts in the soil; this can cause water to move out of the roots and away from the plants. As a result leaves can become dehydrated and take on a burnt look. The leaves in the plants given nitrogen fertiliser were observed to have leaves with ‘burnt’ looking edges that were not observed in the control or the rhizobium inoculant group (See table 2).

Figure 2 shows that the percentage dry to wet weight of the plants inoculated with rhizobia bacteria was the greatest at 33.48% while the nitrogen fertiliser group was 31.06% and the control group was the lowest at 30.26%. The rhizobia inoculant group being the greatest is expected, as the plants were taller in height and with the nodules observed on the roots, this would increase its weight. The nitrogen fertiliser group was second with a dry to wet biomass percentage of 31.06%, despite the fact that the average height of the nitrogen fertiliser group was lower than the control group. This may have been due to the fact that the nitrogen fertiliser group was observed to have more leaves and thicker looking roots than the control group due to the excess nitrogen it received (Table 2). It was observed that the control group had the thinnest looking roots, perhaps due to lack of added nitrogen and other nutrients that are important for root formation. The amount of nitrogen in the plant is related to dry weight and therefore the Rhizobium inoculated group had the highest biomass and therefore the greatest amount of nitrogen.

This experiment was reliable from the data obtained in the results. Three pots per group were used and each pot contained three seeds, which gave a large number of test subjects. The average of all the results was calculated, meaning that every plant was accounted for. For the measurement of plant growth, dry weight (biomass) was used instead of just height, which is more reliable in determining plant growth and nitrogen uptake. The percentage of dry weight to wet weight was calculated, which is a better way to measure biomass.

This experiment was valid in that it did test what was set out to be tested, which was to find out whether the fava bean plant grew better with nitrogen fertilizer or with rhizobia inoculation. This was tested as the heights of the plants were measured daily and the biomass was measured at the end of the experiment. All variables were controlled such as seed type and size, pot size, type and amount of soil, water, sunlight, temperature and humidity and a control was used to confirm that the independent variables of rhizobia bacteria and nitrogen fertilizer were the variables that were affecting plant growth.

The same person measured the plants each day with a millimetre ruler and did the wet and dry biomass, which ensured accuracy. The scale used to weigh the plants measured in grams with 2 decimal points making the weight measurements more accurate.

To improve the experiment more plants should be grown and for a longer period of time. Due to the limited amount of time to do the experiment, the plants were grown for only 6 weeks, ideally it would have been better to grow the plants until they reached maturity and produced seeds and then take the wet and dry weights of the mature plants. Using larger pots to grow the plants would be better so they could be further spaced. In the pots used, the seeds were approximately 3 cm apart, fava beans grow better when planted 10-12 cm apart so the crowding may have affected their growth. Making sure the correct concentration of nitrogen fertiliser used for the quantity of plants would be beneficial to ensure excessive amounts were not used as this can affect growth of the plants.

To extend this research, letting the plants grow until they reached maturity would have been ideal to see if there is a significant difference in plant size, biomass and seeds produced. It would be interesting after the experiment to plant fava beans, that have not been inoculated, into the soil that the inoculated beans were planted to see if they are able to form a symbiotic relationship and nodules from the bacteria already present in the soil. Furthermore, measuring the plant nitrogen would be interesting as it is a good indicator of growth of the plant and how well each method provided nitrogen for the plant.

Conclusion

Fava bean plants inoculated with Rhizobia bacteria had better growth, as measured by height and biomass, than plants given nitrogen fertiliser and the control plants.

The control plants were taller in height than the plants with the nitrogen fertiliser however the nitrogen fertiliser group had a greater biomass than the control.

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