BIOLOGY EXTENDED ESSAY

**Research Question:**

**How do different Nitrogen-Phosphorus-Potassium (NPK) ratios (1:1:1, 2:1:1, 2:2:1 and water) in aqueous fertilisers affect growth (as measured through a mitotic index) of the onion (*Allium cepa*), over a 5- week period, in a hydroponics set up?**

*“The important thing is not to stop questioning. Curiosity has its own reason for existing.” – Albert Einstein*

*(Calaprice, 2000)*

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### Abstract

Humanity sees itself as an adaptive branch of life whose existence is fully self-sufficient, a flawed paradigm. We rely on plants as a source of food and medicine to a monumental extent and the resources available to grow these plants, such as space, are limited. Hence, the optimisation of plant growth is importunate to ensuring that our reserves are used efficiently. The onion is the subject of this essay and was chosen because it plays an underappreciated, but historic role in diets and traditional medicine. NPK fertilisers are frequently used to augment plant growth, but their ratios are varied, each theorised to affect plants in different ways, with limited supporting evidence. Hence, the research question generated was, **“How do different NPK ratios (1:1:1, 2:1:1, 2:2:1 and water) in aqueous fertilisers affect the growth of the onion (*Allium cepa*), over a 5 week period, in a hydroponics set up?”**

20 onions bulbs were placed in 4 hydroponics set ups, each holding 5 bulbs and a different solution. This inquiry was explored through the recording of the mitotic indices (%) of onion root tips, taken from bulbs placed in different solutions, over a period of 5 weeks. 1:1:1, 2:1:1 and 2:1:1 NPK solutions were tested due to their prevalent use worldwide and water was used as a control.

The results suggest that a 1:1:1 NPK ratio is optimal for onion growth (the paired t-tests indicated significant differences between the 1:1:1 solution and remaining NPK solutions). The water solution resulted in a high mitotic index similar to that of the 1:1:1 solution, but due the propensity of the water solution to promote rot, it was an ineffective solution for onion growth.

However, further research is required to determine the effect of different NPK ratios on growth when the onions are grown in soil.

# Word Count: 300 words

**Table of Contents**

Abstract i

List of Abbreviations v

List of Figures vi

Chapter 1: Introduction

* 1. [: Background Knowledge 1](#_TOC_250019)
  2. [: Research Question 3](#_TOC_250018)
  3. [: Literature Review 3](#_TOC_250017)
  4. [: Significance and Worthiness of Investigation 4](#_TOC_250016)

Chapter 2: Investigation

* 1. [: Hypothesis 5](#_TOC_250015)
  2. : Preliminary experiment 5
  3. [: Independent Variable 6](#_TOC_250014)
  4. [: Dependent Variable 6](#_TOC_250013)
  5. [: Controlled Variables 7](#_TOC_250012)

Chapter 3: Method

* 1. [: Materials and Apparatus 9](#_TOC_250011)
  2. [:Organisms 10](#_TOC_250010)
  3. [: Experimental method 10](#_TOC_250009)
  4. [: Limitations 12](#_TOC_250008)

Chapter 4: Results

* 1. [: Qualitative Observations 12](#_TOC_250007)
  2. [: Processed Data 13](#_TOC_250006)
  3. [: Statistical Tests 16](#_TOC_250005)

Chapter 5: Discussion

* 1. : Evaluation of Data 19
  2. [: Evaluation of Procedure 21](#_TOC_250004)
  3. [: Conclusion 24](#_TOC_250003)

[References 27](#_TOC_250002)

[Appendices](#_TOC_250001)

Appendix 1.1: Calculation of the masses of compounds required 33

[Appendix 1.2: Raw Data 36](#_TOC_250000)

**List of Abbreviations**

1. ATP: Adenosine Triphosphate
2. d.f: Degrees of freedom
3. DNA: Deoxyribonucleic Acid
4. EU: European Union
5. G1: Gap 1 Interphase
6. KCl: Potassium chloride
7. mRNA: messenger ribonucleic acid
8. N.A: Not Applicable
9. NH4H2PO4: Ammonium dihydrogen phosphate
10. NPK: Nitrogen-phosphorus-potassium
11. NH4NO3: Ammonium nitrate
12. N: Number of samples
13. SD: Standard Deviation
14. S-phase: Synthesis phase
15. *S. rostrata*: *sesbania rostrata* (a tropical West African legume plant)

**List of Figures**

**Figure 1:** A photograph of the experimental set up taken by myself on 24/11/2015 11

**Figure 2**: An onion cell micrograph 11

**Figure 3:** A bar graph displaying how the average mean mitotic index (%) of all replicates varied between the 4 solutions tested, with each series referring to a replicate 16

**Figure 4:** A bar graph displaying how the average mean mitotic index (%) across all replicates varies between the 4 solutions tested, with ± 1 SD 20

# 1: Introduction

### : Background Knowledge

For millennia, plants have been part of the human diet as food or sources of flavour as herbs or spices. Furthermore, plants are used in the production of medicinal products. In 1993, 3% of American adults used medicinal plant products (Briskin, 2000). In 2007, 38.3% of adults in America used complementary and alternative medicine (NCCIH, 2008), the majority of which being plant-based tinctures.

The onion (*Allium cepa*) is commonly utilised for such dietary and medicinal purposes. In fact, onions were possibly traded as long ago as 2,000BC (Pruszewicz, 2015). Hippocrates, the father of medicine, considered that onion had a, “plethora of medical uses.” (Rabinowitch, Brewster, 1989) The Tamils in India have a similar belief (Grubben, 2004). Onions are rich in fructans (compounds that sustain beneficial gastrointestinal bacteria), flavonoids (antioxidants) and organosulfur compounds (anti-inflammatory agents) (NOA, 2011). These benefits may have been the source of the mentioned beliefs. The consequent high demand for onions would be better met by a high rate of onion growth. Hence, I started looking into how onion growth can be optimised, leading to my research into hyperplasia.

Hyperplasia is an increase in cell count, causing an enlargement of a tissue or organ (Mukherjee, 2010). It is a key process for the growth of an organism. The 3 major factors influencing hyperplasia would appear to be potassium, nitrogen and phosphate concentrations.

Nitrogen is used to produce nucleotides that are added to new strands of DNA during the semi conservative replication of DNA in the S-phase of mitosis, when existing chromosomes are duplicated. Nitrogen is also used in the synthesis of polypeptides and proteins that make up kinases, enzymes made of specialised proteins that facilitate ATP production (Ng, 2013). For example, kinase E1 (CDKE1) (Ng, 2013) is indirectly responsible for the creation of the electrochemical proton gradient. This drives oxidative phosphorylation, the process by which a phosphate group is attached to ADP, producing ATP, which releases energy when hydrolysed (Ng, 2013). The energy released from ATP has a variety of uses, including forming phosphodiester bonds between nucleotides in the replication in DNA in the S- phase. Therefore, without nitrogen, a cell would be unable to undergo mitosis, as kinases would be absent.

Potassium promotes the start of the S-phase of mitosis, because a cell needs to be depolarised for this to begin (Sano, 2007). Cell depolarisation, which initiates the S-phase (Sano, 2007), is facilitated by a large cellular concentration of potassium ions (Sano, 2007), which can be created by NPK fertilisers. This also creates a low turgour pressure that facilitates mitosis (Sano, 2007).

Phosphate is an essential component of DNA, as DNA has a phosphate-based backbone that holds the nucleotides in place, and plays an important role in the cell cycle. This is substantiated by the fact that a phosphate deficient environment provokes G1 arrest by degrading the Cln3 kinase that initiates the S-phase of mitosis (Menoyo, 2013). Hence,

phosphate is essential for the growth of an organism by facilitating the progression of the cell cycle.

The importance of the 3 elements led me to investigate the effect of different NPK ratios on the growth of onions. In fact, NPK fertilisers are often used to supplement plant growth. However, they vary in their elemental ratios (N:P:K) and effects of different ratios on growth have not been fully explored, prompting this investigation.

Having explored the importance of these nutrients, I observed that each nutrient influenced mitosis. I therefore decided to calculate the mitotic index of the onions being tested as a measure of growth. This is further justified in section 2.4.

### : Research Question

**How do different NPK ratios (1:1:1, 2:1:1, 2:2:1 and water) in aqueous fertilisers affect the onion growth (*Allium cepa*) over a 5-week period?**

### : Literature review

Multiple studies have shown that using NPK fertilisers can result in a substantial increase in plant growth. Becker and Diekmann’s study indicated that NPK fertilisers increased nutrient turnover (through nitrogen fixation) by *S rostrata* (Becker, Diekmann, 1991). This indicates that the nitrogen stimulated the root nodules, increasing the rate of nitrogen fixation, hence increasing the roots’ efficiency in absorbing nutrients. This increased nutrient turnover. NPK fertiliser has also reportedly been linked to increased okra (a Nigerian crop) yield (Omotoso, 2007). A lack of the nutrients in NPK fertilisers can be

detrimental to growth of photosynthetic organisms (Cechin, 2007). Cechin’s study proved that crops planted in nitrogen deficient mediums had decreased dry masses (Cechin, 2007), due to a lower rate of photosynthesis that impeded growth.

Cechin’s study was chosen as a reference rather than a similar study by Q. Dortch, as Dortch mainly focused on phytoplankton rather than plant species (Dortch, 1985); hence it was not fully relevant to my research question. Furthermore, Cechin’s paper (2007) is more recent than Dortch’s (1985) and more reliable as it considers modern concepts that had replaced older paradigms.

### : Significance and Worthiness of Investigation

Induced cellular hyperplasia has been an interest of mine since I read Siddhartha Mukherjee’s “The Emperor of All Maladies”. Mukherjee details how uncontrolled hyperplasia indubitably results in malignant and metastatic tumours (Mukherjee, 2011). The influence of internal factors, such as elemental concentrations, on cellular mitosis fascinated me.

However, my literature review showed no studies of how NPK ratios influenced growth at the cellular level.

This dearth of research, combined with my interest in hyperplasia, prompted me to investigate how different NPK ratios influence the growth of onions, using hydroponics. I hope that this investigation will promote further research in the field of microbiology (as it explores how elemental ratios influence cellular hyperplasia) and Botany by exploring a new angle to current research on onions. My study utilises hydroponics (growth of a plant in a liquid

medium). This efficient space saving plant growth technique (Hankinson, 2000) is likely a representation of farming in the near future, given the demands of a rising global population, increasing the significance of my results.

I was inspired to explore this research question, as the results could be relevant to farming in third world communities such as those in Nigeria and Tamilnadu. This would potentially allow them to significantly increase their production of onions per unit time and bring greater economic benefits.

# 2: Investigation

### : Hypothesis

I hypothesize that the 2:2:1 and 2:1:1 ratios will produce the highest mean mitotic indices across all replicates, because these 2 ratios have the highest nitrogen contents of all the tested ratios. A higher quantity of available nitrogen would likely promote greater growth per unit time. As nitrate ions would be readily available, they would be used in the synthesis of amino acids, that are linked together in a series of anabolic reactions to form polypeptides that facilitate growth, like the hydrolase enzyme invertase, which catalyses the hydrolysis of sucrose into glucose (Sturm, 1999).

### : Preliminary experiment and research

A preliminary experiment was conducted over 2 weeks to assess if onion bulbs rather than onion seeds would be more suitable for the experiment. The method employed is identical to that in section 3.3, but only 2 pots were used, one with 5 onion bulbs and one with 5 seeds. Both pots

contained 1.5dm3 of 2:1:1 NPK solution. Additionally, one sponge cube supported 1 seed while 4 sponges supported one bulb. The final method used was adjusted in accordance with the findings from this preliminary experiment. Bulbs were used in the final experiment because of considerable difficulty in determining the mitotic index of the seeds, as few cells had developed in the seed (hence the cells had a darkened appearance). Consequently, a mitotic index could not be calculated because the stages of the cell cycle that each cell was in could not be distinguished. Secondly, more sponges cubes were used to support the bulbs as it was observed that the

bulbs would otherwise often become submerged in the solution.

### : Independent Variable

The independent variable was the NPK ratio in each solution. The NPK ratios tested were (when simplified), 1:1:1, 2:1:1 and 2:2:1. The 1:1:1 ratio was used due to its reputation as being the best ratio for “all-purpose,” use (Smith, 2013). The higher nitrogen level ratios (2:1:1 and 2:2:1) were chosen because these ratios are the most common compound fertiliser ratios in the EU (Normile, 2004). Water was chosen as the control solution as it contains no nitrogen, phosphorus or potassium.

### : Dependent Variable

The dependent variable was the mitotic index of the tip of the longest root of each onion. I chose the root as the tissue to be examined because growth hormones such as cytokinin [a hormone that regulates “physiological growth processes,” (Aiken, 1996) throughout a plant] are produced in the

roots and transported throughout the onion (Aiken, 1996). As cytokinins enhance overall onion growth, high root growth would indicate high overall onion growth. This is because larger roots would produce more cytokinin per unit time, hence facilitating increased growth. Therefore, root mitotic indices would be representative of overall onion growth. *Allium cepa* was chosen because of its widespread worldwide use (Courteau, 2011).

### : Controlled Variables

The species of onion used in the experiment was *Allium cepa*. Different

onion species would respond differently to the same NPK ratio, limiting the extent to which the different ratios can be compared with respect to the growth of the tested onions.

The concentration of the prepared fertiliser in each pot was kept constant at 20g/dm3. Variations in concentrations would affect the rate of nutrient uptake by the onions, hence causing different effects on the growth of onions in each solution.

The environment (temperature, pH, moisture) in which the onions were

placed before the start of the experiment was identical for all the onions as they were stored in the same location.

The time at which the micrograph for each sample was carried out was

kept constant (3:00pm each Tuesday); this was to ensure that the gaps between data points were exactly one week.

The buoyancy of all sponges used was the same at the start of the

experiment. By drying the sponges at the start of the experiment, their initial water contents were identical. Hence, their buoyancies were near identical;

therefore, the volume of solution that the roots were initially exposed to was equal for all the onions as they were held by sponges with near equal water saturation.

The pots used for each set up were identical in dimensions. This

ensured that the volume of each pot was identical, so that the roots had the same growth space. If this were varied, the roots would have grown differently.

# 3: Procedure

### : Materials and Apparatus

|  |  |
| --- | --- |
| **Apparatus** | **Uncertainty** |
| 4 pots  (height 4cm and width 41cm) |  |
| Tap water |  |
| Electronic mass balance | ±0.001g |
| Metal spatula |  |
| Metal scalpel |  |
| 250cm3-measuring cylinder | ±0.5cm3 |
| 25cm3 pipette | ±0.06cm3 |
| 2m2 of green polystyrene mesh |  |
| 100cm3 beaker |  |
| Glass rod |  |
| Adhesive duct tape |  |
| Light microscope |  |
| 40 glass slides |  |
| 120 sponge cubes  (1cm x 1cm x 1cm) |  |
| 55.863g of NH4H2PO4 |  |
| 28.878g of KCl |  |
| 5.232 g of NH4NO3 |  |
| 100cm3 of methyl blue solution |  |

### : Organisms

20 onion bulbs were obtained from the Marine Parade wet market.

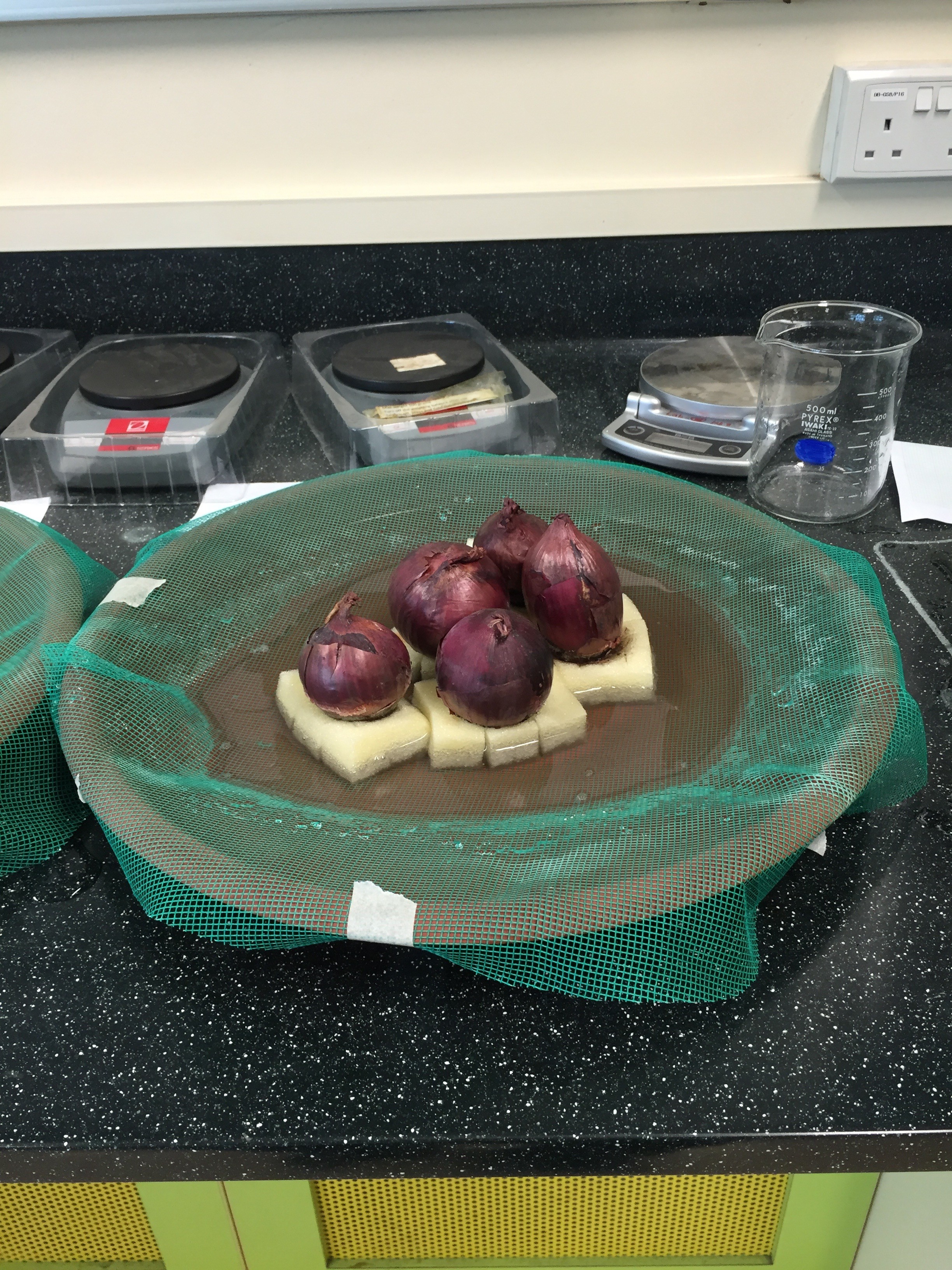
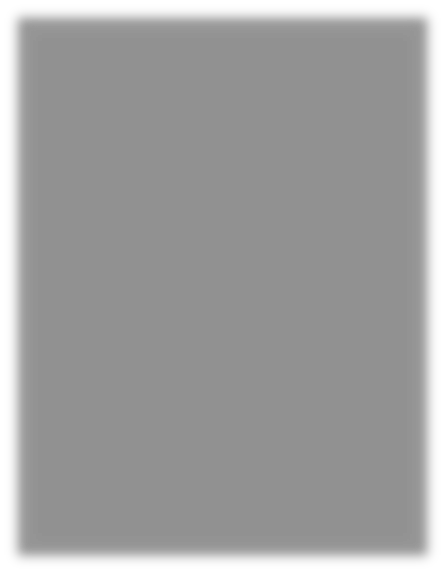
### : Experimental method

Firstly, 4 shallow pots with heights of 4cm and width of 41cm were each filled with 1.50dm3 of tap water using a 250cm3-measuring cylinder (±0.5cm3). Secondly, for each NPK ratio tested, the appropriate masses of NH4H2PO4, KCl and NH4NO3 were calculated as shown in appendix 1.1. A 100cm3 beaker was then placed on an electronic mass balance that was set to zero after the beaker had been placed on it. Using a spatula, sufficient quantities of each compound were added until the desired mass of each compound was reached for a particular ratio. This was repeated for all 3 ratios under investigation. Next, the 4 pots were labelled as “1:1:1,” “2:1:1,” “2:2:1,” and “Water,” for identification. The corresponding solid fertiliser mixtures were added to their respective pots. Once added, the mixture was stirred with a glass rod counter-clockwise over 20 rotations.

Green polystyrene mesh, held taut by duct tape, was used to cover the surface of the pots following the addition of the fertilisers. To secure the onions in place, each onion was placed in 6 fused sponge cubes, each of length 1cm, with a circular hole cut in the centre using a metal scalpel to accommodate the base of the onion bulb. 5 onions were then placed on the mesh covering each pot, to allow for 5 repeats per solution and each were numbered from one to five with tape.

The tip of the longest root was cut from each onion using the metal scalpel before the onions were placed into the sponge base. Each root tip

sample was then crushed with a glass rod rolled forwards and backwards along the length of the root a total of 20 times, with downwards pressure applied. Next, each crushed sample was stained with 1cm3 of dilute methyl blue solution using a pipette (±0.06cm3), to allow the inner structures of the cell to be



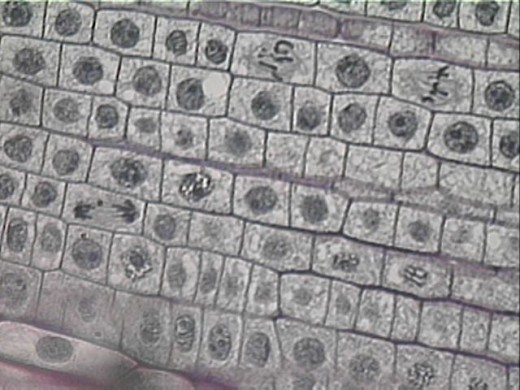
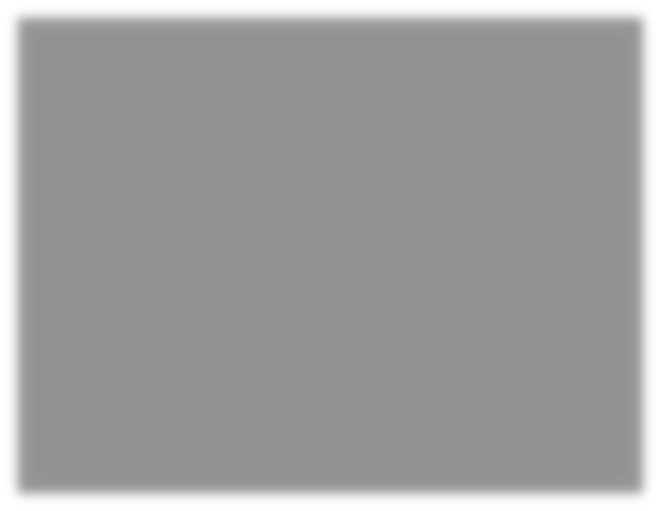
**Figure 1:** A photograph of the experimental set up taken by myself on 24/11/2015

distinguished. The samples were placed between 2 glass slides each, and pressed together. 20 such samples were analysed under a light microscope at x40 magnification and the number of cells in interphase, prophase, metaphase, anaphase and telophase within a 30-cell area were noted.

This sampling and analysis was repeated every week over a 5-week period to determine the mitotic index of the 20 onion root tips across all solutions. 250cm3 of water was added to each pot using the measuring cylinder (±0.5cm3) over 2-day intervals to maintain the concentration of the fertilisers.

The images I observed were similar to that in figure 2, which shows an

image from the magnification of an onion sample.



**Figure 2**: An onion cell micrograph – Baker, The Stages of the Cell Cycle (Mitosis – Interphase and

Prophase), 2013

The stage of mitosis each cell was in was determined by studying their inner structures. In interphase, the nucleus is visible, with uncoiled chromatids. In prophase, the nuclear membrane is dissolved and the chromatids pairs (as chromatids replicate in the S-phase) are visibly supercoiled (denser). During metaphase, mitotic spindles (thread-like extensions from centrioles) push the chromatid pairs to the equator of the cell. In anaphase, the chromatid pairs are separated, with each chromatid on either lateral side of the cell, having been pulled by the mitotic spindles. On either side of the cell, during telophase, nuclear membranes develop around the chromatids that have started to uncoil. To ensure the investigation was objective, I compared each cell to a series of diagrams that depicted each stage (Allott, 2014) and practised identifying which stage of mitosis a cell was in based on its micrograph.

### : Limitations

This investigation is limited to the effect of different NPK ratios on onions grown using hydroponics. Its results do not reflect the effect on onion grown in soil, or using micropropagation techniques.

# 4: Results

### : Qualitative observations

1. 2 onions in the water solution and 1 onion in the 1:1:1 NPK solution sprouted after 3 weeks.
2. The roots in the water solution had become flaccid after 4 weeks.





1. Large root networks formed in the water and 1:1:1 NPK solutions, the largest being in the latter.
2. A white powder was observed on all the pots on the third week – 250cm3 of water was promptly added.

### : Processed Data

Data processing was conducted using the raw data in appendix 1.2.

**Table 1: A table showing the equations utilised in data processing, with sample**

**calculations**

|  |  |
| --- | --- |
| **Equation** | **Sample calculation** |
| 𝑀𝑖𝑡𝑜𝑡𝑖𝑐 𝐼𝑛𝑑𝑒𝑥 = f𝑟𝑒𝑞𝑢𝑒𝑛𝑐(𝑒𝑠 𝑜ƒ 𝑐𝑒𝑙𝑙𝑠 𝑢𝑛𝑑𝑒𝑟&𝑜(𝑛& 𝑚i𝑡𝑜𝑠i𝑠 ⋅ 100%  𝑇𝑜𝑡𝑎𝑙 𝑛𝑢𝑚𝑏𝑒𝑟 𝑜ƒ 𝑐𝑒𝑙𝑙𝑠  (Allott, 2014)  The total frequency of the cells in mitosis is equal to the total number of cells in prophase, metaphase, anaphase and telophase. | *Mitotic index of replicate 4 in the water solution*  2!0!0!0 ⋅ 100%= 6.67%  30 |
| 𝑀𝑒𝑎𝑛 = 𝑡ℎ𝑒 𝑚𝑖𝑡𝑜𝑡𝑖𝑐 𝑖𝑛𝑑𝑒𝑥 𝑜𝑛 𝑒𝑣𝑒𝑟𝑦 𝑤𝑒𝑒𝑘 (𝑓𝑜𝑟 𝑜𝑛𝑒 𝑟𝑒𝑝𝑙𝑖𝑐𝑎𝑡𝑒)  5 | *Mean for the replicate 1 in the water solution*  0.00% + 13.33% + 86.67% + 93.33% + 60.00%  5  = 50.67% |
| 𝑋 − 𝑋 2  𝑆𝑡𝑎𝑛𝑑𝑎𝑟𝑑 𝐷𝑒𝑣𝑖𝑎𝑡𝑖𝑜𝑛 (𝑆𝐷) = (𝑛 − 1)  Where 𝑋 = each mitotic index in the row, 𝑋 = the mean mitotic index, 𝑛 = number of mitotic indices in the row,  represents the sum across all values in the row | *SD for replicate 1 in the water solution*  0 − 50.67 2 + 13.33 − 50.67 2 … + 60.00 − 50.67 2  (5 − 1)  = 42.32 (rounded to 2 decimal places) |

**Table 2: A table showing the mitotic index (%) of each onion root tip immersed**

**in the water solution over 5 weeks including the mean mitotic index for each**

**repeat and standard deviation, using data from tables 1, 5, 9, 13 and 17 in**

**appendix 1.1.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Replicates** | **Number of weeks since the start of the experiment** | | | | | **Mean (%)** |
| **0** | **1** | **2** | **3** | **4** |
| **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** |
| 1 | 0.00 | 13.33 | 86.67 | 93.33 | 60.00 | 50.67 |
| 2 | 0.00 | 10.00 | 90.00 | 83.33 | 73.33 | 51.33 |
| 3 | 3.33 | 0.00 | 70.00 | 76.67 | 0.00 | 30.00 |
| 4 | 6.67 | 26.67 | 36.67 | 100.00 | 80.00 | 50.00 |
| 5 | 0.00 | 40.00 | 73.33 | 100.00 | 86.67 | 60.00 |
| **SD (%)** | 2.98 | 15.56 | 21.16 | 10.38 | 34.96 | 11.06 |

**Table 3: A table showing the mitotic index (%) of each onion root tip immersed**

**in the 1:1:1 NPK solution over 5 weeks including the mean mitotic index for**

**each repeat and standard deviation, using data from tables 2, 6, 10, 14 and 18**

**in appendix 1.1.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Replicates** | **Number of weeks since the start of the**  **experiment** | | | | | **Mean (%)** |
| **0** | **1** | **2** | **3** | **4** |
| **Mitotic Index**  **(%)** | **Mitotic Index**  **(%)** | **Mitotic Index**  **(%)** | **Mitotic Index**  **(%)** | **Mitotic Index**  **(%)** |
| 1 | 0.00 | 63.33 | 63.33 | 100.00 | 60.00 | 57.33 |
| 2 | 3.33 | 43.33 | 80.00 | 100.00 | 70.00 | 59.33 |
| 3 | 0.00 | 30.00 | 46.67 | 56.67 | 43.33 | 35.33 |
| 4 | 10.00 | 70.00 | 60.00 | 100.00 | 93.33 | 66.67 |
| 5 | 0.00 | 53.33 | 56.67 | 53.33 | 63.33 | 45.33 |
| **SD (%)** | 4.34 | 15.91 | 12.16 | 24.68 | 18.17 | 12.41 |

**Table 4: A table showing the mitotic index (%) of each onion root tip immersed**

**in the 2:1:1 NPK solution over 5 weeks including the mean mitotic index for**

**each repeat and standard deviation, using data from tables 3, 7, 11, 15 and 19**

**in appendix 1.1.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Replicates** | **Number of weeks since the start of the experiment** | | | | | **Mean (%)** |
| **0** | **1** | **2** | **3** | **4** |
| **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** |
| 1 | 3.33 | 23.33 | 46.67 | 60.00 | 30.00 | 32.67 |
| 2 | 0.00 | 56.67 | 26.67 | 63.33 | 10.00 | 31.33 |
| 3 | 0.00 | 53.33 | 36.67 | 10.00 | 0.00 | 20.00 |
| 4 | 16.67 | 36.67 | 23.33 | 26.67 | 43.33 | 29.33 |
| 5 | 6.67 | 23.33 | 63.33 | 86.67 | 3.33 | 36.67 |
| **SD (%)** | 6.91 | 15.92 | 16.23 | 30.68 | 18.62 | 6.20 |

**Table 5: A table showing the mitotic index (%) of each onion root tip immersed**

**in the 2:2:1 NPK solution over 5 weeks including the mean mitotic index for**

**each repeat and standard deviation, using data from tables 4, 8, 12, 16 and 20**

**in appendix 1.1.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Replicates** | **Number of weeks since the start of the experiment** | | | | | **Mean (%)** |
| **0** | **1** | **2** | **3** | **4** |
| **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** | **Mitotic**  **Index (%)** |
| 1 | 13.33 | 66.67 | 13.33 | 20.00 | 10.00 | 24.67 |
| 2 | 3.33 | 76.67 | 36.67 | 46.67 | 0.00 | 32.67 |
| 3 | 0.00 | 33.33 | 70.00 | 46.67 | 6.67 | 28.33 |
| 4 | 0.00 | 36.67 | 23.33 | 66.67 | 0.00 | 25.33 |
| 5 | 6.67 | 70.00 | 40.00 | 36.67 | 20.00 | 34.67 |
| **SD (%)** | 5.58 | 20.14 | 21.47 | 17.00 | 8.30 | 4.42 |

**Mean mitotic index (%)**

**Table 6: A table showing the mean mitotic index (%) of each root tip over 5**

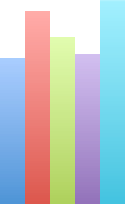
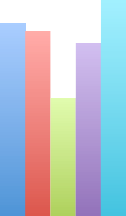
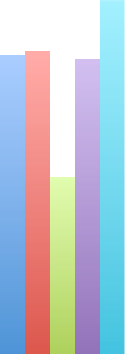
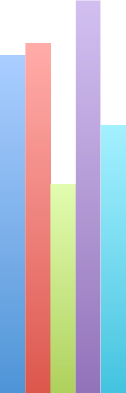
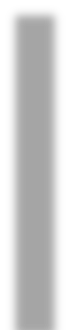
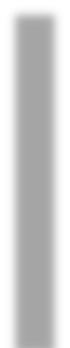
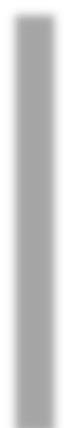
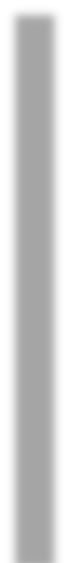
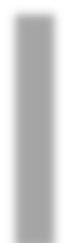
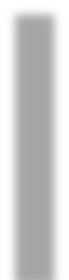
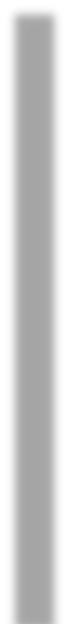
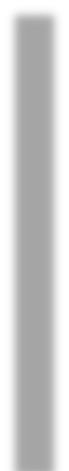
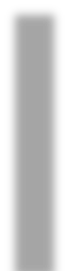
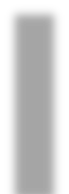
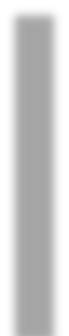
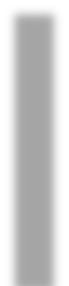
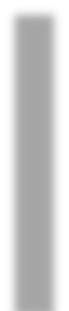
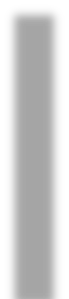
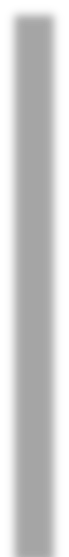
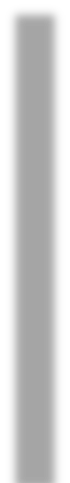
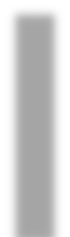
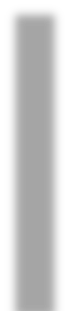
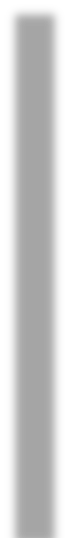
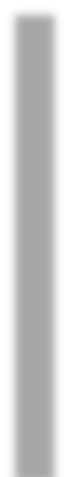
**weeks for the water, 1:1:1, 2:1:1 and 2:2:1 solutions, including means and**

**standard deviation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Replicates** | **Solution** | | | |
| **Water** | **1:1:1** | **2:1:1** | **2:2:1** |
| **Mean mitotic index (%)** | **Mean mitotic index (%)** | **Mean mitotic index (%)** | **Mean mitotic index (%)** |
| 1 | 50.67 | 57.33 | 32.67 | 24.67 |
| 2 | 51.33 | 59.33 | 31.33 | 32.67 |
| 3 | 30.00 | 35.33 | 20.00 | 28.33 |
| 4 | 50.00 | 66.67 | 29.33 | 25.33 |
| 5 | 60.00 | 45.33 | 36.67 | 34.67 |
| **Mean (%)** | 48.40 | 52.80 | 30.00 | 29.13 |
| **SD (%)** | 11.06 | 12.41 | 6.20 | 4.42 |

**Figure 3:** A bar graph displaying how the average mean mitotic index (%) of all replicates varied between the 4 solutions tested, with each series referring to a replicate

### : Statistical Tests



80

70

60

50

40

30

20

Series1 Series2 Series3 Series4

Series5

10

0

Water

1;1;1

2;1;1

2;2;1

Two-tailed t-tests were used to assess if the mitotic indices of the onions grown in certain solutions were statistically higher (*p* ≤ 0.05) than the mitotic indices of the onions grown in different solutions. Two-tailed t-tests were chosen because they test if one set is statistically greater or lower,

numerically, than another. One-tailed t-tests test for a statistically significant difference between data sets, unlike two-tailed t-tests. Hence, two-tailed t- tests were chosen for use in this investigation.

**HO (null hypothesis): The mean mitotic index of set A is greater than of set B**

The following descriptive statistics were produced using StatPlus software, with all values rounded to 3 decimal places.

HO is rejected if t ≥ the critical t value.

**Table 7: A table of descriptive statistics for the water, 1:1:1, 2:1:1 and 2:2:1**

**solutions**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **N** | **Range**  **(%)** | **Minimum**  **(%)** | **Maximum**  **(%)** | **SD**  **(%)** | **Variance**  **(%2)** |
| Water | 5 | 30.000 | 30.000 | 60.000 | 11.061 | 122.354 |
| 1:1:1 | 5 | 31.340 | 35.330 | 66.670 | 12.414 | 154.125 |
| 2:1:1 | 5 | 16.670 | 20.000 | 36.670 | 6.202 | 38.459 |
| 2:2:1 | 5 | 10.000 | 24.670 | 34.670 | 4.421 | 19.549 |

**Table 8: A table of continued descriptive statistics for the water, 1:1:1, 2:1:1 and**

**2:2:1 solutions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Solution** | **Mean (%)** | **Skewness** | **Skewness Standard Error** | **Kurtosis** | |
| **Statistic** | **Standard**  **Error** |
| Water | 48.400 | -0.956 | 0.707 | 2.773 | 0.750 |
| 1:1:1 | 52.798 | -0.395 | 0.707 | 1.782 | 0.750 |
| 2:1:1 | 30.000 | -0.799 | 0.707 | 2.542 | 0.750 |
| 2:2:1 | 29.134 | 0.224 | 0.707 | 1.392 | 0.750 |

**Table 9: One-tailed t-test statistics for the first set of tests**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable Pairs** | **Mean**  **(%)** | | **Variance**  **(%2)** | | **N** | | **Degrees of freedom**  **(d.f)** | **Critical t-value** |
| **A** | **B** | **A** | **B** | **A** | **B** |
| 1:1:1(A) -  2:1:1(B) | 52.798 | 30.000 | 154.125 | 38.459 | 5 | 5 | 8 | 2.306 |
| 1:1:1(A) -  2:2:1(B) | 52.798 | 29.134 | 154.125 | 19.549 | 5 | 5 | 8 | 2.306 |
| 2:1:1(A) -  2:2:1(B) | 30.000 | 29.134 | 38.459 | 19.549 | 5 | 5 | 8 | 2.306 |

**Table 10: One-tailed t-test results for the first set of tests**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable Pairs** | **t-value** | **Is HO rejected?** | **Significance (*p*)** |
| 1:1:1(A) - 2:1:1(B) | 3.673 | Yes | 0.010 > 𝑝 > 0.002 |
| 1:1:1(A) - 2:2:1(B) | 4.015 | Yes | 0.010 > 𝑝 > 0.002 |
| 2:1:1(A) - 2:2:1(B) | 0.254 | No | 1.000 > 𝑝 > 0.500 |

**Table 11: One-tailed t-test statistics for the second set of tests**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable Pairs** | **Mean**  **(%)** | | **Variance**  **(%2)** | | **N** | | **Degrees of freedom**  **(d.f)** | **Critical t-value** |
| **A** | **B** | **A** | **B** | **A** | **B** |
| Water (A) -  2:1:1(B) | 48.400 | 30.000 | 122.354 | 38.459 | 5 | 5 | 8 | 2.306 |
| Water (A) -  2:2:1(B) | 48.400 | 29.134 | 122.354 | 19.549 | 5 | 5 | 8 | 2.306 |
| 1:1:1(A) -  Water (B) | 52.798 | 48.400 | 154.125 | 122.354 | 5 | 5 | 8 | 2.306 |

**Table 12: One-tailed t-test results for the second set of tests**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable Pairs** | **t-value** | **Is HO rejected?** | **Significance (*p*)** |
| Water (A) -  2:1:1(B) | 3.244 | Yes | 0.010 > 𝑝 > 0.005 |
| Water (A) -  2:2:1(B) | 3.616 | Yes | 0.020 > 𝑝 > 0.010 |
| Water (A) -  1:1:1(B) | 0.591 | No | 1.000 > 𝑝 > 0.500 |

# 5: Discussion

### : Evaluation of results

**All mitotic indices referred to in section 5 refer to the mean mitotic index across all replicates.**

The processed data clearly indicates that the solution used in growing onions has a significant impact on the extent to which hyperplasia occurs (Figure 4).

The water solution yielded a very high mitotic index (48.40%), the second highest of all tested solutions. However, the data had high SD (11.061%), demonstrating that there was significant variation in the mitotic index between replicates.

The onions grown in the 1:1:1 solution had the highest mitotic index (52.798%) out the 3 solutions tested, but had high SD (12.414%), demonstrating inconsistency in the mitotic indices. However, the lowest mean mitotic index for a repeat was only 35.33%, which is similar to the maximum mitotic index for the 2:1:1 and 2:2:1 solutions.

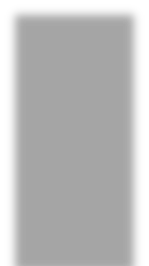
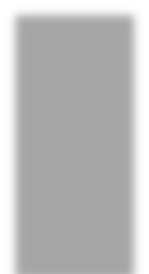
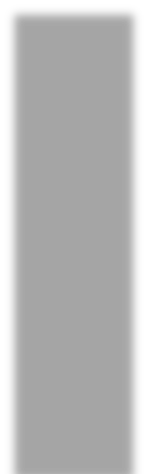
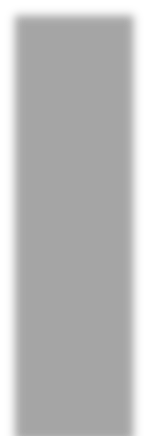
The mitotic index of the root tips placed in the 2:1:1 solution had a low value of 30.00% and low SD (6.202%), suggesting that these onions had limited growth due to the fertiliser used. This is synonymous with previous studies on 2:1:1 NPK ratios (Ghaffoor, 2003).

The 2:2:1 solution also resulted in limited root growth as shown by the relatively low mitotic index (29.134), and had low SD (4.421).

The statistical tests indicated a statistically significant difference in the mitotic index between the 2:1:1 and 1:1:1 solution; the 2:2:1 and 1:1:1 solution; the water and 2:1:1 solution and the water and 2:2:1 solution (tables

9 and 11). This delineates the fact that the water and 1:1:1 solutions beget higher mitotic indices than 2:1:1 and 2:2:1 solutions.

However, the data also indicates no statistically significant difference between the mitotic indices of onions placed in the 1:1:1 and water solution, and those of the 2:1:1 and 2:2:1 solutions. Hence, it can be determined that both high nitrogen fertilisers decrease onion growth to the same extent, and additionally, that the 1:1:1 and water solutions analogously increase the mitotic index of onions, as depicted in figure four.



70

60

50

40

30

20

10

0

Water

1;1;1

2;1;1

2;2;1

**Type of solution of used**

**M3an mitotic index across all repeats over 4 weeks (%)**

**Figure 4:** A bar graph displaying how the average mean mitotic index (%) across all replicates varies between the 4 solutions tested, with ± 1 SD.

My results are substantiated by Brown, who found that high nitrogen NPK fertilisers result in reduced seed yield (Brown, 1980).

### : Evaluation of procedure

The table below contains a list of experimental limitations and realistic modifications to reduce the impact of the limitations.

**Table 13: A table of limitations and modifications to address those limitations**

|  |  |
| --- | --- |
| **Limitation** | **Modification** |
| 1. There was no waste disposal system in the hydroponics set ups, possibly allowing for the build up of metabolic waste, impeding onion  growth. | A pump could have been used remove waste solution and replace it with newly produced solution. |
| 2. The lack of use of NH4NO3 in the 1:1:1 and 2:2:1 solutions resulted in  an absence of nitrate ions that possibly reduced growth relative to the 2:1:1 solution that contained nitrate ions. | Other compounds could have been used such that each solution contained the same aqueous ions and compounds. |
| 3. The ages of onions used were varied, as the onions had been  grown at different times, hence their ability to grow further was varied as shown by the relatively high SD in the 1:1:1 and water solution. | The onions could have been planted at the same time and their bulbs could have been harvested after one month for use in the study. |
| 4. The temperature of the | The air-conditioning should be left |

|  |  |
| --- | --- |
| **Limitation** | **Modification** |
| surroundings was not maintained. The air conditioning was always switched off at 4pm and switched on at 7am. Hence, the temperature was not constant. This change in temperature possibly affected nitrate reductase activity in the onions. This enzyme catalyses protein translation (Armstrong, 1998); hence, an increase in temperature would facilitate increased production of these proteins because increasing temperature increases nitrate reductase activity as it has a high optimum temperature (Armstrong, 1998). This would consequently result in a higher mitotic index due to increased protein availability for the S-phase of mitosis. | on overnight, to ensure the temperature remained constant at 23OC. |
| 5. The quantity of water added was possibly insufficient to compensate for evaporation from the pots, as  white powder was visible on the | The appropriate amount of water should be added on a daily basis to counter the calculated rate of  evaporation based on the surface |

|  |  |
| --- | --- |
| **Limitation** | **Modification** |
| meshes in the third week. This possibly caused minor variations in each pot’s fertiliser concentration (the concentration increased when the volume of solution decreased due to evaporation). This possibly altered the rate of nutrient uptake by the onions and hence would have affected hyperplasia in the onions. | area of the pots, temperature, wind speed and humidity. |
| 6. Potentially, there was parallax error in the experiment as it is likely  that some of the cells were mistakenly identified by accident, hence the recorded mitotic index would be different from the true mitotic index. | The number of cells in each stage of mitosis could be counted a second time to ensure that no mistakes were made. |
| 7. The pots were not aerated, limiting the availability of oxygen to the roots. This reduced their ability  to respire due to smaller  concentrations of oxygen that consequently reduced ATP | An air pump could be installed to pump oxygen into the solutions. |

|  |  |
| --- | --- |
| **Limitation** | **Modification** |
| concentration, because the mitochondria in the root hair cells had fewer terminal electron acceptors in the form of oxygen. |  |

### : Conclusion

In conclusion, the NPK solution used as an aqueous fertiliser has a significant impact on growth in onions. In hydroponics, a 1:1:1 NPK fertiliser should be used as a solution because it induces high onion growth. This has disproven my initial hypothesis that the 2:2:1 and 2:1:1 solutions facilitate optimum onion growth. This investigation has also confirmed previous research on NPK ratios in fertilisers and their effect on growth (Brown, 1980; Ghaffoor, 2003; Chapin, 1987), particularly in high nitrogen ratios.

The two high nitrogen ratios had the lowest mitotic indices. The differences between their means were statistically insignificant. This can be attributed to the negative effects of the high nitrogen content of both fertilisers. Large amounts of ATP were used to absorb nitrate and ammonium ions in the surplus conditions (Smith, 2013). Chapin substantiates this, asserting that absorbing nitrogen makes up a “significant,” and, “predominant fraction of the total energy a plant consumes.” (Chapin, 1987) This therefore reduced the quantity of available ATP to be mobilised in the transcription of mRNA that trigger mitosis-inducing genes (Schneider, 2003). Therefore, statistically, the mitotic index was significantly higher in the water and 1:1:1 solution compared to the 2:1:1 and 2:2:1 solution, as the water and 1:1:1 solutions did not have

excess nitrogen. Thus, onions in these 2 solutions sprouted unlike those in the remaining solutions.

The 1:1:1 solution had the highest mean mitotic index as it contained the necessary nutrients for a high rate of mitosis in sufficient quantities such that all the nutrients could be absorbed without depleting significant amounts of ATP. However the difference in mitotic indices between the water and 1:1:1 solution was statistically insignificant. This shows that tap water is a useful solution in the hydroponic growth of onions, since it induces growth akin to a 1:1:1 NPK solution, across five weeks. This is possibly due to the multiple nutrients present in tap water, such as nitrate and phosphate ions, albeit in small concentrations (PUB, 2015), which potentially enhanced onion growth. However, in the water solution, rot had formed on the roots in the final two weeks. This reduced the number of living cells and, consequently, the onions’ mitotic indices in the last two weeks of the experiment. This can be attributed to the shallow root network that had limited access to air, whereas the root networks of the onions in the 1:1:1 solution were closer to the surface. Hence, the roots in the water solution had little exposure to air, hindering their ability to respire, promoting apoptosis (cell death) in root cells and reducing the mitotic index.

I have high confidence in my results, due to the low uncertainty of the apparatus used and minor impact of the limitations of the experiment on the results from the NPK solutions. These results are possibly of high significance to improving onion cultivation techniques in third world countries, such as Nigeria and India. However, this investigation is limited to demonstrating the effectiveness of these fertilisers in the context of hydroponics set-ups. A new

area for investigation would be to uncover which NPK fertilisers is most suitable when onions are farmed conventionally in soil. Additionally, natural fertilisers could be compared to NPK fertilisers, with respect to their ability to induce onion growth.

## References

Aiken. R. M. and Smucker A.J.M., 1996. *ROOT SYSTEM REGULATION OF*

*WHOLE PLANT GROWTH 1.* Annual review of phytopathology 34, no. 1 : 325-346.

Allott. A., 2014. *Oxford IB Diploma Programme Biology Course Companion*, Oxford University Press, Oxford

Armstrong. D., 1998, *Better Crops with Plant Food – Potassium for Agriculture*, International Plant Nutrition Institute, Better Crops, Volume 82, No 3, pages 3-40, Georgia, USA, https://ipni.net/ppiweb/bcrops.nsf/$webindex/EA503A5EC59681B3852

568C700157B00/$file/98-3.pdf, date of access 18/11/2015, 2:09pm

Baker. R., 2013, *Stages of the Cell Cycle - Mitosis (Interphase and Prophase)*, Hubpages, Life Sciences, <http://hubpages.com/education/Stages-of-the-Cell-Cycle-Mitosis-Part->

1-of-2#, date of access 12/12/15 7:36 pm.

Becker. M., Diekmann., 1991. *Effect of NPK on growth and nitrogen fixation of Sesbania rostrata as a green manure for lowland rice (Oryza sativa*

*L.*). *Plant and soil*, *132*(1), pp.149-158, [http://link.springer.com/article/10.1007/BF00011021#page-1,](http://link.springer.com/article/10.1007/BF00011021#page-1) 22/11/15,

10:43am

Briskin, D.P., 2000. Medicinal plants and phytomedicines. *Linking plant biochemistry and physiology to human health*. Plant Physiology, 124(2), pp.507-514,

https://[www.plantphysiol.org/content/124/2/507.full.pdf+html,](http://www.plantphysiol.org/content/124/2/507.full.pdf%2Bhtml) date of

access 26/9/2015, 7:46pm

Brown, K.R., 1980. *Seed production in New Zealand ryegrasses*: II. Effects of N, P, and K fertilisers. New Zealand journal of experimental agriculture, *8*(1), pp.33-39.

Calaprice, A., 2000. *The Expanded Quotable Einstein*, pp.262. Princeton: Princeton University Press

Cechin, I., de Fátima Fumis, T., 2004. *Effect of nitrogen supply on growth and photosynthesis of sunflower plants grown in the greenhouse*. *Plant Science*, *166*(5), pp.1379-1385,

[http://faculty.kfupm.edu.sa/CHEM/thukair/Reading/RA7/76.pdf,](http://faculty.kfupm.edu.sa/CHEM/thukair/Reading/RA7/76.pdf) date of

access 23/11/15, 7:40am

Courteau., 2011. *Brief summary of allium cepa*. Encyclopaedia of Life, [http://eol.org/pages/1084354/details,](http://eol.org/pages/1084354/details) date of access 26/3/2016

Chapin, F.S., Bloom, A.J., Field, C.B. and Waring, R.H., 1987. *Plant responses to multiple environmental factors*. Bioscience, 37(1), pp.49- 57.

Dortch, Q.; Clayton, J. R.; Thoresen, S. S.; Cleveland, J. S.; Bressler, S.

L.; Ahmed, S. I., 1985. *Nitrogen storage and use of biochemical indices to assess nitrogen deficiency and growth rate in natural plankton populations*, Journal of Marine Research, Volume 43, Number 2,

pp. 437-464(28)

Ghaffoor, A., Jilani, M.S., Khaliq, G. and Waseem, K., 2003. *Effect of Different NPK Levels on the Growth and Yield of Three Onion (Allium сера L.) Varieties*. Asian Journal of Plant Sciences, 2(3), pp.342-346, [http://scialert.net/fulltext/?doi=ajps.2003.342.346&org=11,](http://scialert.net/fulltext/?doi=ajps.2003.342.346&org=11) date of

access, 9/12/15, 10:04am

Grubben, G.J., 2004. *Vegetables* (Vol.2), Prota, https://books.google.com.sg/books?hl=en&lr=&id=6jrlyOPfr24C&oi=fnd

&pg=PA6&dq=Grubben+onion+use&ots=DqBdnNKt1Y&sig=36Fljc67Q

FQeT4jLTP8nqAbanbg#v=onepage&q=Grubben%20onion%20use&f=f

alse, page 45, date of access 9/11/2015, 2:12pm

Rabinowitch, D., J. L. Brewster., 1989. *Onions and Allied Crops: Biochemistry Food Science Minor Crops* (Vol.3). CRC Press, https://books.google.com.sg/books?hl=en&lr=&id=VFGYQcYkCYoC&oi

=fnd&pg=PA2&dq=(Onions+and+Allied+Crops:+Biochemistry+Food+S

cience+Minor+Crops,+Volume+3+By+Haim+D.+Rabinowitch,+J.+L.+Br

ewster)&ots=3gPPBbX-

A7&sig=63WV5d3gzgKo\_BiJMvrD1DMbsow#v=onepage&q=(Onions%

20and%20Allied%20Crops%3A%20Biochemistry%20Food%20Science

%20Minor%20Crops%2C%20Volume%203%20By%20Haim%20D.%2

0Rabinowitch%2C%20J.%20L.%20Brewster)&f=false, date of access

22/11/15, 11:05am

Hankinson. A., *A Hydroponics Lesson Plan*, 2000, The Growing Edge International, Best of The Growing Edge International, New Moon Publishing, Corvallis, USA, 2005, pages 316, 317, https://books.google.com.sg/books?id=lZD95wlLhxIC&pg=PA318&lpg=

PA318&dq=why+do+plants+grow+best+in+1:1:1+solution&source=bl&

ots=1m3VWbd0Q4&sig=hS7A6syfPpjJ5Q3sZ\_IH\_j-

dp8Q&hl=en&sa=X&ved=0ahUKEwiZn7eL0drJAhWHjo4KHcvIBagQ6A

EIGjAA#v=onepage&q=why%20do%20plants%20grow%20best%20in

%201%3A1%3A1%20solution&f=false, date of access 13/12/15,

4:23pm

International Baccalaureate (IB)., 2014, *IB Chemistry Data booklet* (first assessment 2016), Table 6, page 6, IB, 2014, Geneva

Menoyo. S, Ricco, N., Bru, S., Hernández-Ortega, S., Escoté, X., Aldea, M. and Clotet, J., 2013. Phosphate-activated cyclin-dependent kinase stabilizes G1 cyclin to trigger cell cycle entry. *Molecular and cellular biology*, *33*(7), pp.1273-1284.

Mukherjee. S., 2010. *The Emperor of All Maladies: A Biography of Cancer,*

Simon and Schuster, pages 34-59

National Onion Association (NOA)., 2011, *Health Properties of* Onions, NOA, Colorado, USA, https://[www.onions-usa.org/media/view/14/Health-](http://www.onions-usa.org/media/view/14/Health-)

Properties-of-Onions, date of access 24/11/15, 11:08am

Normile. M., Leetmaa. S., 2004, *US-EU food and agriculture comparisons*. US Department of Agriculture, Agriculture and Trade Report. USDA, Washington., https://books.google.com.sg/books?id=OQLdCLc93ysC&pg=PA42&lpg

=PA42&dq=most+common+NPK+ratio&source=bl&ots=m\_mDm5k\_cz

&sig=ofQUgtQD9in3-

Ayjyxg111YMSy8&hl=en&sa=X&ved=0ahUKEwjosJCX0NfJAhXHTI4K

HZc0BE84ChDoAQhMMAg#v=onepage&q=most%20common%20NP

K%20ratio&f=false, date of access 13/12/15, 10:43pm

Ng. S., Giraud. E., Duncan. O., Law. S.R., Wang. Y, Xu. L, Narsai. R, Carrie.

C, Walker. H, Day. D.A and Blanco, N.E., 2013. *Cyclin-dependent kinase E1 (CDKE1) provides a cellular switch in plants between growth and stress responses*. Journal of Biological Chemistry, *288*(5),

pp.3449-3459.

Omotoso. S., Shittu., 2007. *Effect of NPK Fertilizer Rates and Method of Application on Growth and Yield of Okra (Abelmoschus esculentus (L.) Moench) at Ado-Ekiti Southwestern Nigeria*. International Journal of Agricultural Research, 2: 614-619, [http://scialert.net/fulltext/?doi=ijar.2007.614.619,](http://scialert.net/fulltext/?doi=ijar.2007.614.619) date of access

23/11/15 7:23 am

Pruszewicz. M., 2015. *3 Cheers for the Onion,* BBC Magazine*,* [http://www.bbc.com/news/magazine-30549150,](http://www.bbc.com/news/magazine-30549150) date of access

22/11/15 11:01am

Public Utilities Board (PUB) Singapore., 2015, *Drinking Water Quality Report,*

[http://www.pub.gov.sg/general/watersupply/Pages/DrinkingWQReport.](http://www.pub.gov.sg/general/watersupply/Pages/DrinkingWQReport)

aspx, date of access 15/12/15 12:19pm

Sano, T., Becker, D., Ivashikina, N., Wegner, L.H., Zimmermann, U, Roelfsema, M.R.G., Nagata, T. and Hedrich, R., 2007. *Plant cells must pass a K+ threshold to re‐enter the cell cycle*. *The Plant Journal*, *50*(3), pp.401-413.

Schneider, D., Gourse, R., 2004. *Relationship between Growth Rate and ATP Concentration in Escherichia coli A BIOASSAY FOR AVAILABLE CELLULAR ATP*. *Journal of Biological Chemistry*, *279*(9), pp.8262- 8268, [http://www.jbc.org/content/279/9/8262.short,](http://www.jbc.org/content/279/9/8262.short) date of access

14/12/15

Smith. H., 2013, *Resources*, NPK Industries, [http://npk-](http://npk-/)

industries.com/plant\_nutrition.html, date of access 10/12/15, 9:25pm

Sturm, A., 1999. Invertases. Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant physiology*, *121*(1), pp.1-8.

The National Centre for Complementary and Integrative Health (NCCIH), 2008, *The Use of Complementary and Alternative Medicine in the United* States, US Department of Health and Human Services, https://nccih.nih.gov/research/statistics/2007/camsurvey\_fs1.htm, date

of access 26/9/15, 8:23pm

**Appendices**

### Appendix 1.1: Calculation of masses of compounds required

I first generated the nitrogen to phosphorus to potassium ratios in each solution by creating a molar ratio system whereby the number of moles of nitrogen atoms number of moles of phosphorus and potassium atoms were compared. NH4H2PO4 has 1 nitrogen atom and 1 phosphorus atom, and KCl has 1 potassium atom, therefore one mole of both compounds would produce one mole of nitrogen and phosphorus as well as one mole of potassium respectively, therefore producing a 1:1:1 solution when the number of moles of each compound is identical. Hence, the number of moles of each compound would be equal to the moles of the investigated atoms. NH4NO3 was only used in the 2:1:1 solution, as its inclusion allows for double the moles of nitrogen atoms compared to potassium atoms, without doubling the number of phosphorus atoms (because if the number of moles of NH4H2PO4 was to be doubled, the number of moles of phosphorus and nitrogen atoms would double). For the 2:2:1 solution, there must be 2 moles of nitrogen and phosphorus for every mole of potassium and this can be accomplished if the number of moles of NH4H2PO4 is double that of KCl.

The appropriate masses of NH4H2PO4, KCl and NH4NO3 needed for each NPK ratio were calculated through the mathematical equations explained in appendix 1.1.

**Table 1: A table displaying the number of moles and mass of each compound**

**used to generate each NPK ratio**

|  |  |  |  |
| --- | --- | --- | --- |
| **NPK ratio** | **1:1:1** | **2:1:1** | **2:2:1** |
| 𝒙 **value (moles)** | 0.158 | 0.131 | 0.098 |
| **Number of moles of NH4H2PO4 used**  **(moles)** | 0.158 | 0.131 | 0.197 |
| **Number of moles of KCl used (moles)** | 0.158 | 0.131 | 0.098 |
| **Number of moles of**  **NH4NO3 used (moles)** | N.A | 0.065 | N.A |
| **Mass of NH4H2PO4 used (g) (±0.001g)** | 18.176 | 15.029 | 22.658 |
| **Mass of KCl used (g)**  **(±0.001g)** | 11.797 | 9.739 | 7.342 |
| **Mass of NH4NO3 used (g)(±0.001g)** | N.A | 5.232 | N.A |

As the ratio used in this experiment is a molar ratio, the number of moles of each compound needed for each ratio is found. This was done in the manner listed below.

1. The total mass of the fertiliser mixture is set to 30g.
2. The unknown value 𝑥 is set to be the number of moles of each compound required.
3. Using the formula

𝑚𝑎𝑠𝑠 (𝑔) = 𝑚𝑜𝑙𝑒𝑠 𝑚𝑜𝑙 ⋅ 𝑅𝑒𝑙𝑎𝑡𝑖𝑣𝑒 𝑓𝑜𝑟𝑚𝑢𝑙𝑎 𝑚𝑎𝑠𝑠 (𝑔𝑚𝑜𝑙!1), the number of moles of each compound needed to create a 1:1:1 NPK ratio is examined in the following manner.

1. The relative formula mass of each compound is found by adding up the masses of the individual elements in the compound, with the relative atomic masses of each element taken from the IB Chemistry Data booklet.

a. *Example Calculation for the relative formula mass of KCl (39.10 + 35.45 = 74.55)* (IBO, 2014)

5. First, the equation (74.55)(𝑥) + (115.04)(𝑥) = 30 (IBO, 2014) was solved such that the required number of moles of each compound needed for the generation of a 1:1:1 NPK ratio was found. Following this, the mass of each compound required was found by multiplying the number of moles required by its corresponding relative molecular mass.

*a. Example calculation for the 1:1:1 NPK ratio*

(74.55)(𝑥) + (115.04) (𝑥) = 30

189.59𝑥 = 30

𝑥 = 0.158 (𝑟𝑜𝑢𝑛𝑑𝑒𝑑 𝑡𝑜 3 𝑑𝑒𝑐𝑖𝑚𝑎𝑙 𝑝𝑙𝑎𝑐𝑒𝑠)

𝑀𝑎𝑠𝑠 𝑜𝑓 𝐾𝐶𝑙 𝑟𝑒𝑞𝑢𝑖𝑟𝑒𝑑 = 0.158 ⋅ 74.55 = 11.797𝑔

*Mass of NH4H2PO4 required* = 0.158 ⋅ 115.04 = 18.176𝑔

6. For 2:1:1, the equation (74.55)(𝑥) + (115.04)(𝑥) + (80.09)(𝑥) =

2

30 (IBO, 2014) was solved.

7. For 2:2:1, the equation (74.55)(𝑥) + (115.04)(2𝑥) = 30 (IBO, 2014)

was solved.

8. The results of these calculations are shown in table 1 of this appendix.

### Appendix 1.2: Raw Data

**Table 1: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area at the start of the experiment across the 5 onions**

**immersed in the water solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 30 | 0 | 0 | 0 | 0 |
| 2 | 30 | 0 | 0 | 0 | 0 |
| 3 | 29 | 0 | 0 | 1 | 0 |
| 4 | 28 | 2 | 0 | 0 | 0 |
| 5 | 30 | 0 | 0 | 0 | 0 |

**Table 2: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area at the start of the experiment across the 5 onions**

**immersed in the 1:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 30 | 0 | 0 | 0 | 0 |
| 2 | 29 | 1 | 0 | 1 | 0 |
| 3 | 30 | 0 | 0 | 0 | 0 |
| 4 | 27 | 0 | 1 | 0 | 0 |
| 5 | 30 | 0 | 0 | 0 | 0 |

**Table 3: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area at the start of the experiment across the 5 onions**

**immersed in the 2:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 29 | 0 | 0 | 1 | 0 |
| 2 | 30 | 0 | 0 | 0 | 0 |
| 3 | 30 | 0 | 0 | 0 | 0 |
| 4 | 25 | 0 | 0 | 0 | 0 |
| 5 | 28 | 1 | 0 | 1 | 0 |

**Table 4: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area at the start of the experiment across the 5 onions**

**immersed in the 2:2:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 26 | 1 | 1 | 0 | 2 |
| 2 | 29 | 0 | 1 | 0 | 0 |
| 3 | 30 | 0 | 0 | 0 | 0 |
| 4 | 30 | 0 | 0 | 0 | 0 |
| 5 | 28 | 5 | 1 | 1 | 0 |

**Table 5: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 1 week after the start of the experiment across the 5**

**onions immersed in the water solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 26 | 3 | 0 | 1 | 0 |
| 2 | 27 | 0 | 3 | 0 | 0 |
| 3 | 30 | 0 | 0 | 0 | 0 |
| 4 | 22 | 4 | 2 | 0 | 2 |
| 5 | 18 | 4 | 7 | 0 | 1 |

**Table 6: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 1 week after the start of the experiment across the 5**

**onions immersed in the 1:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 11 | 14 | 0 | 3 | 2 |
| 2 | 17 | 6 | 3 | 2 | 2 |
| 3 | 21 | 2 | 0 | 5 | 2 |
| 4 | 9 | 13 | 2 | 1 | 5 |
| 5 | 14 | 4 | 1 | 7 | 4 |

**Table 7: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 1 week after the start of the experiment across the 5**

**onions immersed in the 2:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 23 | 2 | 4 | 1 | 0 |
| 2 | 13 | 7 | 4 | 5 | 1 |
| 3 | 14 | 3 | 5 | 6 | 2 |
| 4 | 19 | 4 | 2 | 5 | 0 |
| 5 | 23 | 0 | 3 | 3 | 1 |

**Table 8: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 1 week after the start of the experiment across the 5**

**onions immersed in the 2:2:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 10 | 6 | 0 | 9 | 5 |
| 2 | 7 | 5 | 8 | 8 | 2 |
| 3 | 20 | 0 | 7 | 3 | 0 |
| 4 | 19 | 2 | 0 | 8 | 1 |
| 5 | 9 | 11 | 2 | 8 | 0 |

**Table 9: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 2 weeks after the start of the experiment across the 5**

**onions immersed in the water solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 4 | 16 | 2 | 7 | 1 |
| 2 | 3 | 0 | 21 | 4 | 2 |
| 3 | 9 | 14 | 4 | 2 | 1 |
| 4 | 19 | 3 | 5 | 2 | 1 |
| 5 | 8 | 1 | 9 | 12 | 0 |

**Table 10: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 2 weeks after the start of the experiment across the 5**

**onions immersed in the 1:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 11 | 10 | 5 | 4 | 0 |
| 2 | 6 | 6 | 10 | 3 | 5 |
| 3 | 16 | 6 | 8 | 0 | 0 |
| 4 | 12 | 7 | 6 | 4 | 1 |
| 5 | 13 | 2 | 8 | 6 | 3 |

**Table 11: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 2 weeks after the start of the experiment across the 5**

**onions immersed in the 2:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 16 | 14 | 0 | 0 | 0 |
| 2 | 22 | 4 | 0 | 4 | 0 |
| 3 | 19 | 3 | 4 | 2 | 2 |
| 4 | 23 | 1 | 5 | 1 | 0 |
| 5 | 11 | 8 | 6 | 4 | 1 |

**Table 12: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 2 weeks after the start of the experiment across the 5**

**onions immersed in the 2:2:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 26 | 0 | 3 | 0 | 1 |
| 2 | 19 | 5 | 3 | 3 | 0 |
| 3 | 9 | 6 | 7 | 5 | 3 |
| 4 | 23 | 4 | 2 | 0 | 1 |
| 5 | 18 | 0 | 8 | 4 | 0 |

**Table 13: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 3 weeks after the start of the experiment across the 5**

**onions immersed in the water solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 2 | 13 | 0 | 14 | 1 |
| 2 | 5 | 21 | 1 | 3 | 0 |
| 3 | 7 | 18 | 3 | 0 | 2 |
| 4 | 0 | 24 | 1 | 4 | 1 |
| 5 | 0 | 7 | 19 | 2 | 2 |

**Table 14: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 3 weeks after the start of the experiment across the 5**

**onions immersed in the 1:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 0 | 9 | 13 | 1 | 7 |
| 2 | 0 | 18 | 7 | 1 | 4 |
| 3 | 13 | 1 | 8 | 0 | 8 |
| 4 | 0 | 2 | 11 | 8 | 9 |
| 5 | 14 | 4 | 8 | 3 | 1 |

**Table 15: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 3 weeks after the start of the experiment across the 5**

**onions immersed in the 2:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 12 | 13 | 0 | 7 | 6 |
| 2 | 11 | 15 | 0 | 4 | 0 |
| 3 | 27 | 1 | 2 | 0 | 0 |
| 4 | 22 | 7 | 0 | 1 | 1 |
| 5 | 4 | 6 | 11 | 5 | 4 |

**Table 16: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 3 weeks after the start of the experiment across the 5**

**onions immersed in the 2:2:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 24 | 3 | 2 | 0 | 1 |
| 2 | 16 | 6 | 1 | 4 | 3 |
| 3 | 16 | 5 | 6 | 0 | 3 |
| 4 | 10 | 0 | 5 | 11 | 4 |
| 5 | 19 | 8 | 3 | 0 | 0 |

**Table 17: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 4 weeks after the start of the experiment across the 5**

**onions immersed in the water solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 12 | 6 | 4 | 1 | 7 |
| 2 | 8 | 3 | 12 | 5 | 2 |
| 3 | 30 | 0 | 0 | 0 | 0 |
| 4 | 6 | 9 | 2 | 0 | 13 |
| 5 | 4 | 19 | 6 | 0 | 1 |

**Table 18: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 4 weeks after the start of the experiment across the 5**

**onions immersed in the 1:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 12 | 8 | 7 | 3 | 0 |
| 2 | 9 | 13 | 0 | 0 | 8 |
| 3 | 17 | 2 | 2 | 4 | 3 |
| 4 | 2 | 8 | 11 | 4 | 5 |
| 5 | 11 | 5 | 12 | 0 | 2 |

**Table 19: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 4 weeks after the start of the experiment across the 5**

**onions immersed in the 2:1:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 21 | 1 | 3 | 4 | 1 |
| 2 | 27 | 0 | 3 | 0 | 0 |
| 3 | 30 | 0 | 0 | 0 | 0 |
| 4 | 17 | 1 | 10 | 1 | 1 |
| 5 | 29 | 0 | 1 | 0 | 0 |

**Table 20: A table showing the number of cells in each stage in mitosis in**

**a 30 cell area 4 weeks after the start of the experiment across the 5**

**onions immersed in the 2:2:1 NPK solution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Replicates | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis | Number of cells in stage of  mitosis |
| Interphase | Prophase | Metaphase | Anaphase | Telophase |
| 1 | 27 | 1 | 0 | 0 | 2 |
| 2 | 30 | 0 | 0 | 0 | 0 |
| 3 | 28 | 1 | 0 | 1 | 0 |
| 4 | 30 | 0 | 0 | 0 | 0 |
| 5 | 24 | 2 | 3 | 1 | 0 |