# Biology Extended Essay

Investigation of anti-cancer and anti-genotoxic properties of different concentrations of ***Azadirachta indica*** leaf extract by using Allium Cepa Root Tip Assay

How does the concentration of ***Azadirachta indica*** (neem) leaf extract (0.000, 0.002, 0.004, 0.006, 0.008 and 0.010 $g/cm^3$) without or with $50cm^3$ of 3% hydrogen peroxide ($H_2O_2$) affect the Mitotic Index (MI) and the abnormal mitotic index(A-MI) in the root tips of *Allium cepa*, the duration of the experiment (24h), the species of the onion used (*Allium cepa var. aggregatum*), and the initial root length of the onion (1 inch) the same?

**Word count: 3999**

1 INTRODUCTION ..... 4

1.1 MOTIVATION BEHIND INVESTIGATION ..... 4
1.2 RESEARCH QUESTION ..... 4
1.3 BACKGROUND INFORMATION ..... 5
1.4 HYPOTHESIS ..... 9

2 METHODOLOGY ..... 10

2.1 MATERIALS AND APPARATUS LIST ..... 10
2.2 METHOD OF CONTROL OF VARIABLES ..... 11
2.3 PRELIMINARY TRIALS ..... 12
2.4 EXPERIMENTAL PROTOCOL ..... 13
2.5 ENVIRONMENTAL, SAFETY AND ETHICAL ISSUES ..... 16

3 RESULTS ..... 17

3.1 QUALITATIVE DATA ..... 17
3.2 TABLE OF FORMULAE USED ..... 20
3.3 QUANTITATIVE AND PROCESSED DATA ..... 21
3.4 VISUAL REPRESENTATION USING GRAPHS ..... 22
3.5 DATA ANALYSIS: STATISTICAL TESTS ..... 24

4 DISCUSSION ..... 29

5 EVALUATION AND EXTENSIONS ..... 32

6 CONCLUSION ..... 34

7 BIBLIOGRAPHY ..... 35

8 APPENDIX ..... 40

8.1 BACKGROUND INFORMATION ..... 40
8.2 METHODOLOGY ..... 40
8.3 RAW DATA FOR THE INVESTIGATION ..... 42

**8.4 ANOVA TEST AND TUKEY TEST (FULL) .......... 46**

**9 PRELIMINARY RESULTS FOR OTHER ABNORMALITIES AFTER 24H .......... 49**

**9.1 QUALITATIVE DATA .......... 49**
**9.2 QUANTITATIVE DATA .......... 49**
**9.3 DISCUSSION .......... 50**

**10 PRELIMINARY RESULTS FOR INVESTIGATING MI AFTER 48H .......... 51**

**10.1 RAW DATA FOR PART 2 .......... 51**
**10.2 PROCESSED DATA .......... 52**
**10.3 DATA ANALYSIS .......... 53**
**10.4 DISCUSSION .......... 58**

# 1 INTRODUCTION

## 1.1 MOTIVATION BEHIND INVESTIGATION

Recently, increasing rates of cancer is the leading cause of death[1]. However, the current medicine given to treat cancer like chemotherapy has many side effects like nausea and pain[2]. In India, the Neem plant is an herb believed to cure cancer and even prevent relapses. However, laboratory researches show that excessive amounts of neem can cause adverse effects[3]. I was curious to find out if this was true, what amount of neem would be just right to prevent cancer while minimising adverse effects.

Research has shown high Reactive oxygen species (ROS) levels can cause cancer by causing genotoxicity and DNA damage[4]. J. Spencer et al. showed more DNA stand breaks with increasing concentration of $H_2O_2$[4]. Hence, I decided to investigate how neem prevents oxidative damage caused by $H_2O_2$.

## 1.2 RESEARCH QUESTION

How does the concentration of ***Azadirachta indica*** (neem) leaf extract (0.000, 0.002, 0.004, 0.006, 0.008 and 0.010 $g/cm^3$) without or with $50cm^3$ of 3% hydrogen peroxide ($H_2O_2$) affect the Mitotic Index (MI) and the abnormal mitotic index(A-MI) in the root tips of *Allium cepa*, the duration of the experiment (24h), the species of the onion used (*Allium cepa var. aggregatum*), and the initial root length of the onion (1 inch) the same?

## 1.3 BACKGROUND INFORMATION

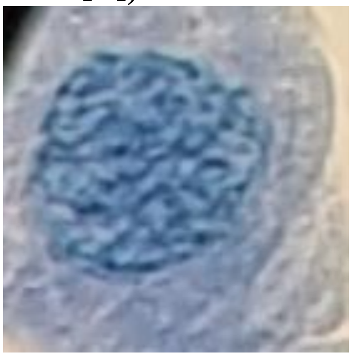
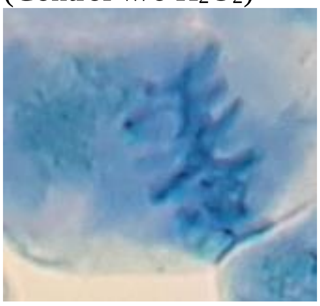
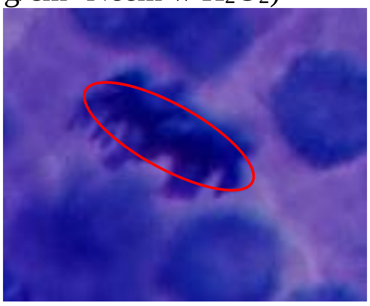
### *1.3.1 Cell cycle regulation and abnormal cell division*

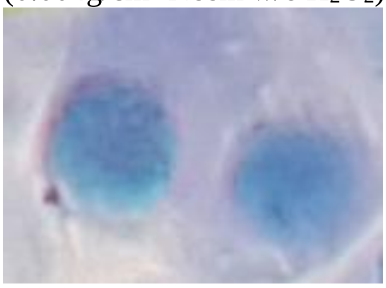
Mitosis, a process where the cell separates its duplicated DNA into two new nuclei, is highly regulated to prevent fatal mistakes. The cell cycle is regulated by many kinases (enzymes that attach phosphate groups to specific target proteins to activate them) in a cascade reaction. Cyclins drive the cell cycle by associating themselves with other enzymes, called cyclin-dependent kinases (CDKs). When a cyclin binds to the CDKs, it makes a functional enzyme and modifies target proteins.

If DNA is damaged, it is detected and the cell is halted at $G_1$ stage of mitosis. A protein called p53 responds to DNA damage. This protein, a tumor suppressor, stops the cell at $G_1$, activating DNA repair proteins, allowing DNA repair to occur. If DNA is not repairable, p53 will trigger programmed cell death[5–7]. However, mutations in the p53 gene, can cause the cell to enter mitosis instead of self-destructing. This cell is cancerous, duplicating rapidly and lacking differentiated functions[8]. In humans cancerous cells can lead to fatalities as it can interfere with normal cellular functions[9].

### *1.3.2 Abnormal vs normal mitotic cells*

*Table 1: Normal vs Abnormal Mitotic Figures[10] (self-taken)*

| | Normal | Abnormal |
|---|---|---|
| Prophase | A: Normal Prophase (Control w/o $H_2O_2$)<br><br>Condensing chromatids forming chromosomes, spread out evenly throughout the nucleus | B: Vacuolated nucleus at prophase (0.002g/$cm^3$ Neem w $H_2O_2$)<br><br>Patches of lighter area and this is known as vacuoles |
| | There are many theories surrounding the cause of nuclear vacoulation (i.e true holes, nuclear invagination by cytoplasm, glycogen accumalation etc)[11,12] | |
| Metaphase | C: Normal Metaphase (Control w/o $H_2O_2$)<br><br>Chromosomes, distinct from each other, lining up in the equotorial plane of the cell | D: Sticky Metaphase (0.002 g/$cm^3$ Neem w $H_2O_2$)<br><br>Chromosomes stick to each other (darker band) and distinct chromosomes cannot be seen |
| | Sticky metaphase could be due to too much adhesin holding the chromosomes together, causing it to strongly adhere to each other[13,14]. | |

| | | |
|---|---|---|
| Anaphase | E: Normal Anaphase (Control w/o $H_2O_2$)  Two distinct portions of DNA and they are migrating to the poles of the cell | F: Anaphase bridge (0.002g /$cm^3$ w $H_2O_2$)  There is a "bridge" |
| | The "Bridge" is probably due to the adhesion of chromosomes and abnormal splitting of the centromeres[13,14]. | |
| Telophase | G: Telophase-Cytokenisis (0.004g/$cm^3$ Neem w/o $H_2O_2$)  Two equally shaped nucleus | H: unequal division, causing micro nuclei at telophase (0.004g/$cm^3$ neem w $H_2O_2$)  One small nucleus and one large nucleus and this is called the micro nuclei |
| | Abnormality could be due to interference with chemicals involved in proper splitting DNA[13]. | |

### *1.3.3 Reactive Oxygen Species*

Reactive oxygen species (ROS) contain oxygen atom(s) more reactive than normal $O_2$ gas. ROS are produced naturally during cellular respiration and photosynthesis in the mitochondria, chloroplast and peroxisomes[15]. High levels of ROS can cause oxidative stress on cells, DNA damage and lead to cancer[16,17].

$H_2O_2$ increases cell proliferation by mediating the MAPK/ERK cascade[16]. $H_2O_2$ results in a signaling cascade where one protein activates another which activates another protein. The cascade reaches the final protein called ERK (MAPK) which is translocated to the nucleus where it expresses certain genes leading to cell proliferation[18].

In this investigation, $H_2O_2$ was used as the ROS. $H_2O_2$ can pass through the cell membrane quite easily due to its permeability. Once, it reaches the cell, it reacts with the ions in the cell to produce a highly reactive OH• radical. This OH• radical then goes onto attack the DNA and cause damage to the DNA[19].

### *1.3.4 Azadirachta indica*

*Figure 1: The Neem Tree used*

The neem tree, contains phytochemicals like Quercetin, Rutin, and Nimbolide, which contribute to anti-cancer and anti-genotoxic properties[20,21]. Quercetin and Rutin are flavonoids which gets oxidized in place of DNA molecules, preventing DNA from being oxidized[22].

Nimbolide disrupts Mitochondrial Outer Membrane Permeabilization (MOMP) by disrupting the Bcl-2 family of proteins responsible for cell apoptosis[21,23,24], resulting in apoptosis. Nimbolide also disrupts the Nrf2-KEAP1 complex, releasing more Nrf2. Nrf2 is a protein that regulates gene expression of antioxidant response[25]. However, too much Nrf2 can allow cancer cell survival and proliferation[21,24,26]. Nimbolide also disrupts other pathways, like the MAPK (ERK ½) cascade that cause cell proliferation. Hence nimbolide can cause MI to fall[24].

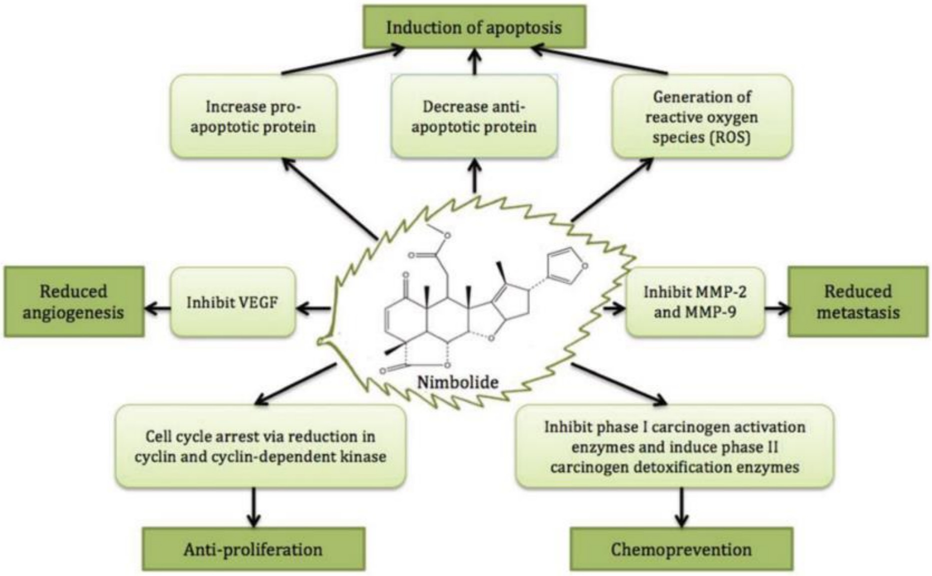



*Figure 2: Properties of Nimbolide*[24]

## 1.4 HYPOTHESIS

In the set-up with $H_2O_2$, nimbolide has to reduce oxidative stress. In the process it could get metabolized. Hence little nimbolide is left to reduce cell proliferation. However in the set-up without $H_2O_2$, all the nimbolide is being used to reduce cell proliferation. Therefore, **as the concentration of neem increases, the MI in the set-up with $H_2O_2$ decreases to a less extent compared to the MI in set-up without $H_2O_2$.**

In the set-up with $H_2O_2$, higher levels of abnormalities are present as compared to the set up without $H_2O_2$. Hence, **as the concentration of neem increases, A-MI for the set-up with $H_2O_2$ decreases with a larger extent compared to the A-MI in set-up without $H_2O_2$.**

# 2 METHODOLOGY

## 2.1 MATERIALS AND APPARATUS LIST

*Table 2: Apparatus and materials list*

| Apparatus | Quantity/Size/Brand for 1 replicate | Uncertainty |
|---|---|---|
| Plastic Cups | 14 | - |
| Sewing thread | 1 spool | - |
| $10cm^3$ Measuring cylinder | 1 | $\pm 0.2cm^3$ |
| $50\ cm^3$ Measuring cylinder | 1 | $\pm 0.5cm^3$ |
| $250cm^3$ Measuring cylinder | 1 | $\pm 2cm^3$ |
| Microscope | 1 | - |
| Slide and coverslip | 14 | - |
| Razor Blade | 1 | - |
| Watch glasses | 1 | - |
| Forceps | 1 | - |
| Digital Camera | 1 | - |
| Ruler | 1 | 0.1cm |
| Stone mortar and pestle | 1 set | - |
| Tissue paper | 1 roll | - |
| Glass stirrer | 1 | - |
| Permanent marker for labelling | 1 | - |
| Plastic dropper | 1 | - |
| Weighing Scale | 1 | ± 0.01g |

*Table 3: Chemicals List*

| Chemicals / liquids / organisms | Quantity for 1 replicate |
|---|---|
| 6% Hydrogen Peroxide | $150\ cm^3$ |
| Deionized water bottle | 2 |
| 0.5% aqueous toluidine blue dropper | 1 |
| 0.1M HCl dropper | 1 |
| ***Azadirachta indica*** leaves | 4.50g |
| ***Allium Cepa*** | 14 |

## 2.2 METHOD OF CONTROL OF VARIABLES

**Independent Variable:** Concentration of ***Azadirachta indica*** leaves extract(0.002, 0.004, 0.006, 0.008, 0.010g/cm$^3$)

***Azadirachta Indica*** leaves have anti-cancer and anti-oxidant properties. However, an experiment done by researchers in the university of Agriculture in Nigeria found out that concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 g/cm$^3$ causes chromosomal aberrations[3]. Hence, 10 to 100 times less the concentration was used (0.002, 0.004, 0.006, 0.008, 0.010 g/cm$^3$) to see if there is any difference in the results and to find the ideal concentration that gives benefits and minimizes genotoxicity.

**Dependent Variable:** MI and A-MI of *Allium cepa* root tip

MI quantifies the number of cells undergoing mitosis given by the formula: $\frac{Cells\ in\ Mitosis}{Total\ number\ of\ cells} \times 100\%$ . A very high MI can indicate rapidly growing cells. Chromosomal aberrations (detected under the light microscope) is quantified using the abnormal mitotic index given by the formula: $\frac{Cells\ undergoing\ abnormal\ mitosis}{Total\ number\ of\ mitotic\ cells} \times 100\%$ . Both high MI and A-MI suggests the presence of cancerous cells, hence, chosen to test for anti-cancer properties of different concentrations of neem. By counting the total number of cells in one microscopic view of at least 2 roots from 1 onion and count the number of normal and abnormal mitotic cells, MI and A-MI can be found.

**Control Variable (1):** Species of *Allium cepa*

Different species have different number of chromosomes and rate of cell proliferation at the root tip. Hence keeping the species the same is important. Keep the *Allium cepa* to one species – *Allium cepa var. aggregatum*.

**Control Variable (2):** The duration the root tips are immersed in the solution

The duration has to be the same across all the set-ups to ensure that any variance in MI between set-ups is not due to the root tips being exposed to the mixture longer or shorter than the other set-ups. Make sure that the MI is taken after 24h strictly

**Control Variable (3):** Initial length of root tip

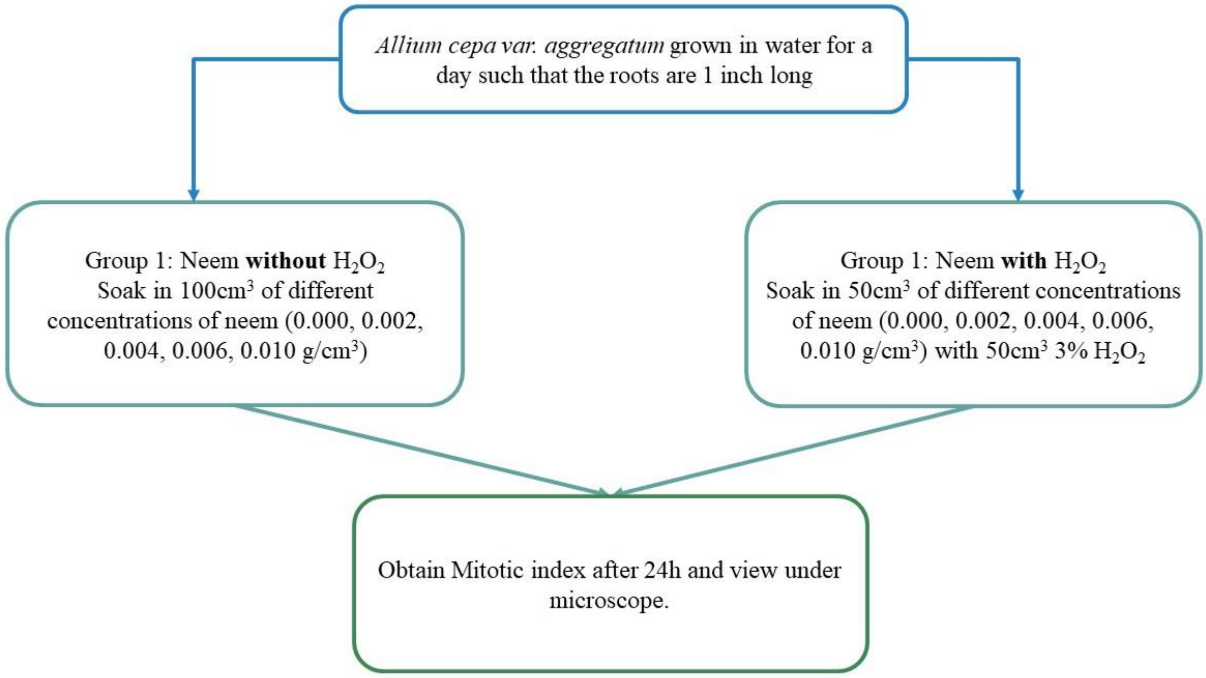
Different lengths of roots will have different MIs different  area exposed to the solution. Hence any change in the initial length of root tip may cause MIs to be different and the results may be affected as well. Make sure that the initial length of root tip is 1 inch.

### 2.3 PRELIMINARY TRIALS

In the preliminary trials, data was taken after 24h, 72h, and 120h. However, these timing did not give good results as exposure to $H_2O_2$ was too long for and it caused the roots to die off. Also, timings of 72h and 120h caused the onions to rot and allowed fungal growth. Hence the experiment was cut short to 24h and 48h.

In the preliminary trials, 6% $H_2O_2$ used was too concentrated causing roots of the onion in one set-up (the one with no leaf extract) to completely dissolve into the hydrogen peroxide. Hence the concentration was reduced to 3%.

## 2.4 EXPERIMENTAL PROTOCOL




Drawn using MS PowerPoint

### 2.4.1 *Preparation*

1. Grow the onion roots to about 1 inch in water(details in Section 8.2.1) which takes 24h.
2. Weigh 4.50g of Neem leaves using a weighing scale.
3. Using a stone mortar and pestle, grind the neem leaves using a stone mortar and pestle. This method was adopted as it is tradition to grind the leaves and drink its juice.
4. Use 5.0cm$^3$ of deionized water measured using a 10cm$^3$ measuring cylinder to grind the neem leaves.
5. After grinding all the leaves, top the solution volume to 450cm$^3$ using a measuring cylinder.
6. To make the 3% $H_2O_2$ solution, combine 150cm$^3$ 6% $H_2O_2$ stock solution and 150cm$^3$ water, both measured using 200cm$^3$ measuring cylinders. Always use fresh stock as hydrogen peroxide is photosensitive[27,28].

### 2.4.2 *Experimental protocol*

1. Prepare the test and control set-ups according to the table below.

*Table 4: Volumes for each independent variable*

| Neem concentration / g/cm$^3$ | | Volume of neem / cm$^3$ | Volume of distilled Water / cm$^3$ | 3% $H_2O_2$ Stock / cm$^3$ | Total volume / cm$^3$ |
|---|---|---|---|---|---|
| Group 1: Without $H_2O_2$ | 0.000 | 0 | 100 | - | 100 |
| | 0.002 | 20 | 80 | - | 100 |
| | 0.004 | 40 | 60 | - | 100 |
| | 0.006 | 60 | 40 | - | 100 |
| | 0.008 | 80 | 20 | - | 100 |
| | 0.010 | 100 | 0 | - | 100 |
| Group 2: With $H_2O_2$ | 0.000 | 0 | 50 | 50 | 100 |
| | 0.002 | 10 | 40 | 50 | 100 |
| | 0.004 | 20 | 30 | 50 | 100 |
| | 0.006 | 30 | 20 | 50 | 100 |
| | 0.008 | 40 | 10 | 50 | 100 |
| | 0.010 | 50 | 0 | 50 | 100 |

2. Soak the onions in the respective cups according to table 4.
3. After 24h, take out the tip of the root (2mm).
4. Soak 2 root tips in 0.1M HCl for 2 min. Rinse the root tips.
5. Soak 2 root tips in toluidine blue dye for 2 min. Rinse the root tips.
6. Place the root tips on a slide and put a drop of water on it. Place a cover slip on it followed by a tissue paper on it. Using the flat portion of the hand, squash the root tip.

*Figure 3: Flat portion of the hand*

7. Observe the root under the microscope and count the MI.
8. 2 roots from one onion is considered 1 replicate. Do 10 replicates for each of the independent variables.

## 2.5 ENVIRONMENTAL, SAFETY AND ETHICAL ISSUES

There are no ethical issues in this investigation.

Even though plastic cups make the experiment easier to carry out, it increases the use of plastic which is not environmentally friendly. As such, cups can be washed, dried and recycled.

When there is extensive fungal infection of the onion, use gloves to handle the onion and dispose the set-up appropriately in the biohazard waste. At high concentrations of $H_2O_2$, it can cause irritation of the skin[27]. However, since a low concentration was used, it is safe. Keep $H_2O_2$ away from naked flames[27,28]. Cutting the root tip using a blade and while using glassware can cause injury. Be careful while using the blade and do not leave both the blade and glassware in unsupervised areas to avoid injuries. The dye used is Toluidine Blue is a relatively a safe dye[29].

# 3 RESULTS

## 3.1 QUALITATIVE DATA

Concentration of neem / g/cm$^3$

0.000 0.002 0.004 0.006 0.008 0.010

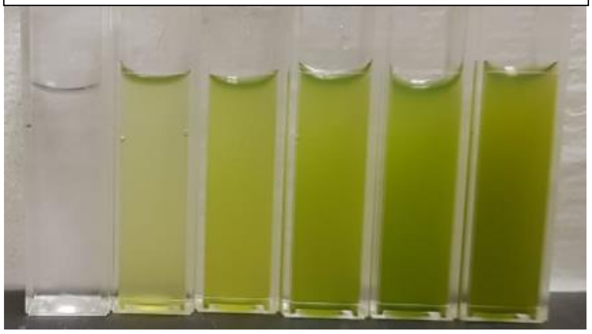


*Figure 4: Neem extract without $H_2O_2$*

As the concentration of neem increases from 0.000 to 0.010g/cm$^3$ (left to right), the intensity of the green color increases.

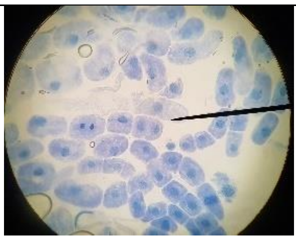
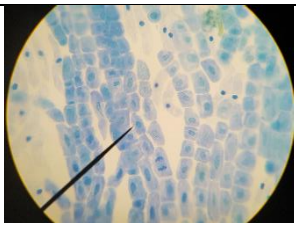
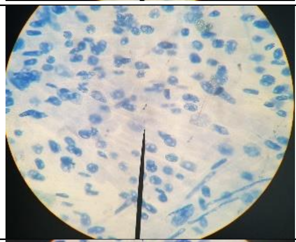
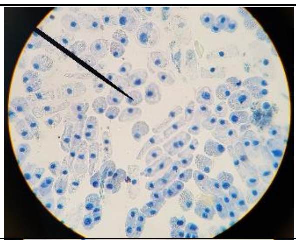
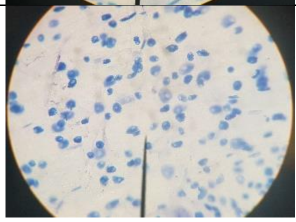
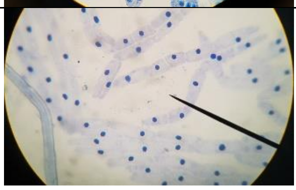
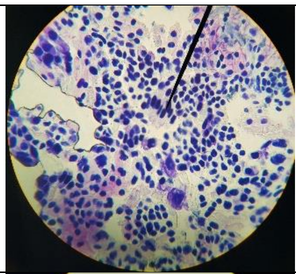
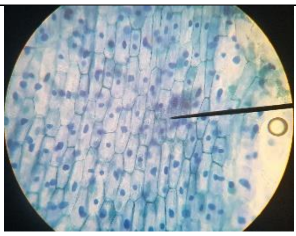
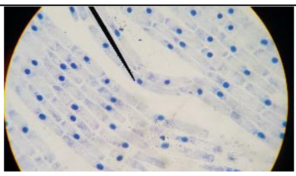
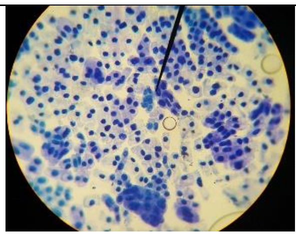
Concentration of neem / g/cm$^3$

0.000 0.002 0.004 0.006 0.008 0.010

*Figure 5: Neem extract reacting with hydrogen peroxide.*

When the hydrogen peroxide (3%) was added, the neem and the hydrogen peroxide started reacting. As the concentration of neem (in g/cm$^3$) increases, the depth of the foam increases, showing increasing vigorous reaction.

*Table 5: Microscopic view of the root tips after the various treatments*

| Neem Concentration / $g/cm^3$ | Neem without $H_2O_2$ | Neem with $H_2O_2$ | Description |
|---|---|---|---|
| 0.000 |  |  | The root exposed to $H_2O_2$ shows a more abnormal elongation of the nuclei, vacuolated cells and cells undergoing abnormal prophase than the root not exposed to $H_2O_2$. |
| 0.002 |  |  | |
| 0.004 |  |  | The root exposed to $H_2O_2$ shows more vacuolated cells and cells undergoing abnormal mitosis than the root not exposed to $H_2O_2$. |
| 0.006 |  |  | The root exposed to $H_2O_2$ still has cells udergoing abnormal mitosis and cells that have vacuolated nucleus. However, the root not exposed to $H_2O_2$ has no cells at mitosis and all cells have a dense ideal nucleus. |
| 0.008 |  |  | |
| 0.010 |  |  | |

| Concentration of neem / g/cm$^3$ | | | | | |
|---|---|---|---|---|---|
| 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |

*Figure 6: Roots of the onions soaked in the set-up without $H_2O_2$ after 24h.*

As the concentration of neem increases, the root get harder.

| Concentration of neem / g/cm$^3$ | | | | | |
|---|---|---|---|---|---|
| 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |

*Figure 7: Roots of the onions soaked in the set-up with $H_2O_2$ after 24h.*

At concentration 0.000g/cm$^3$, the length is much shorter than other concentrations. The roots are also generally softer to touch that the roots from the set-up without $H_2O_2$.

## 3.2 TABLE OF FORMULAE USED

*Table 6: Formulae used*

| For | Formula | Example |
| --- | --- | --- |
| Mean | $\frac{\sum mitotic\ index\ of\ replicates}{10}$ | For control w/o $H_2O_2$ and 0.000 g/cm$^3$ neem,<br>Mean =<br>$\frac{6.63+10.37+11.41+5.39+57.78+12.10+33.55+18.50+17.48+18.07}{10}$<br>= 19.13 (to 2 d.p) |
| Mitotic Index, regardless of abnormality | $\frac{Cells\ in\ Mitosis}{Total\ number\ of\ cells} \times 100\%$ | For control w/o $H_2O_2$ and 0.000 g/cm$^3$ neem,<br>Replicate 1 Root 1<br>$= \frac{28+2+1+0}{313} \times 100\% = 9.90\%$ (to 2 d.p) |
| Abnormal Mitotic Index | $\frac{Cells\ undergoing\ abnormal\ mitosis}{Total\ number\ of\ mitotic\ cells} \times 100\%$ | For control w/o $H_2O_2$ and 0.000 g/cm$^3$ neem,<br>Replicate 1 Root 1<br>$= \frac{2}{31} \times 100\% = 6.67\%$ (to 2d.p) |

## 3.3 QUANTITATIVE AND PROCESSED DATA

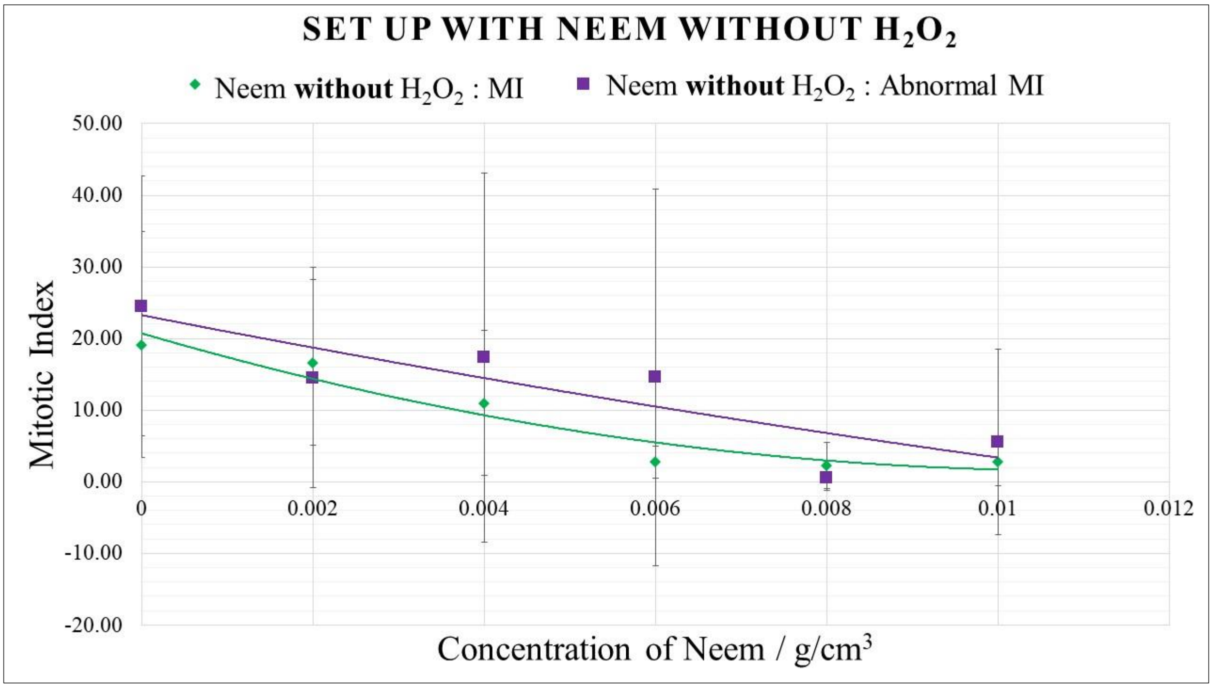
*Table 7: An example of raw data (0.000 g/cm³ neem without $H_2O_2$: Replicate 10)*

| Raw Data | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Root no. | Prophase | Metaphase | Anaphase | Telophase | A-Prophase | A-metaphase | A-Anaphase | A-telophase |
| 1 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2 | 14 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Calculations | | | | | | | | |
| Root no. | Total number of cells in normal mitosis | Total number of cells in Abnormal Mitosis | Total Number of cells | MI-N | MI-A | | | |
| 1 | 15 | 2 | 128 | 13.28 | 11.76 | | | |
| 2 | 15 | 1 | 70 | 22.86 | 6.25 | | | |
| Average | 15 | 2 | 99 | 18.07 | 9.01 | | | |

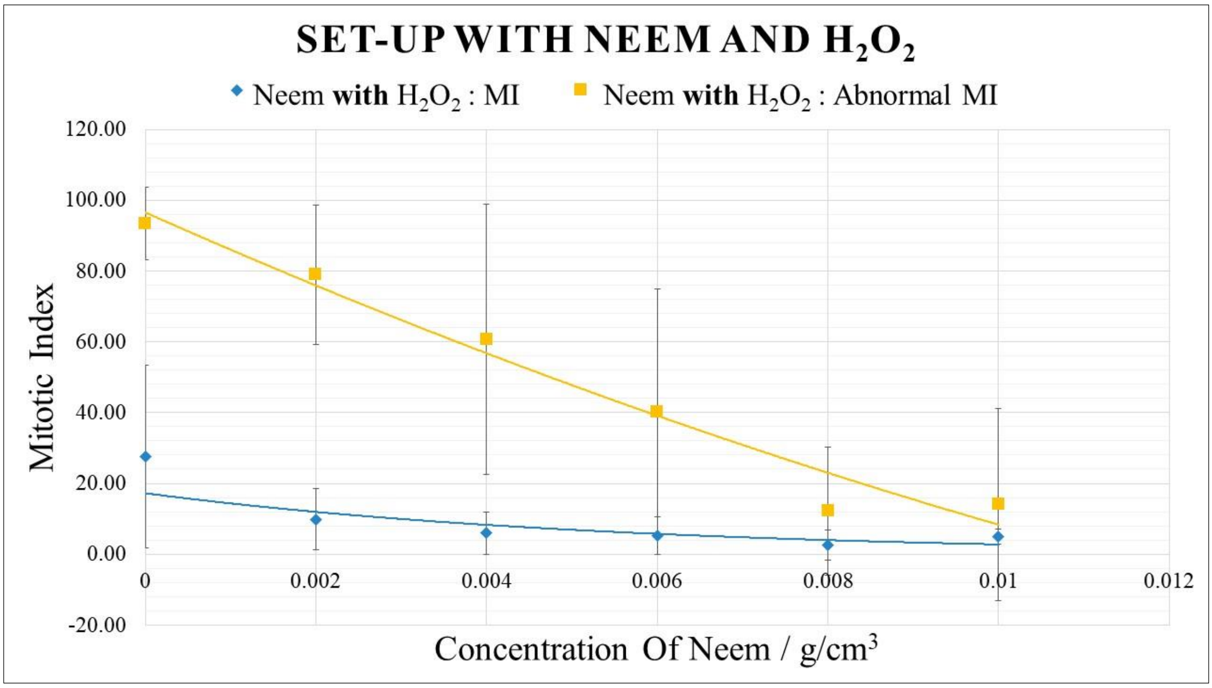
*Table 8: Summary of data for each independent variable*

| | Concentration of neem / g/cm³ | Average number of cells in abnormal mitosis | Average number of cells undergoing abnormal mitosis | Average Total Number of cells | Average MI | Average A-MI |
|---|---|---|---|---|---|---|
| Without $H_2O_2$ | 0.000 | 24.5 | 2.8 | 136 | 19.13 | 24.51 |
| | 0.002 | 28.2 | 3.5 | 184 | 16.66 | 14.54 |
| | 0.004 | 11.2 | 1.1 | 125 | 11.00 | 17.36 |
| | 0.006 | 2.0 | 0.3 | 132 | 2.74 | 14.58 |
| | 0.008 | 2.8 | 0.2 | 134 | 2.25 | 0.58 |
| | 0.010 | 2.2 | 0.5 | 101 | 2.80 | 5.60 |
| | | | | | | |
| With $H_2O_2$ | 0.000 | 1.10 | 23.25 | 114.75 | 27.65 | 93.41 |
| | 0.002 | 3.50 | 14.70 | 181.30 | 9.85 | 78.96 |
| | 0.004 | 0.55 | 5.55 | 134.15 | 6.01 | 60.74 |
| | 0.006 | 2.80 | 3.65 | 143.50 | 5.27 | 40.35 |
| | 0.008 | 2.55 | 1.15 | 135.65 | 2.54 | 12.17 |
| | 0.010 | 5.55 | 0.55 | 164.85 | 4.94 | 14.07 |

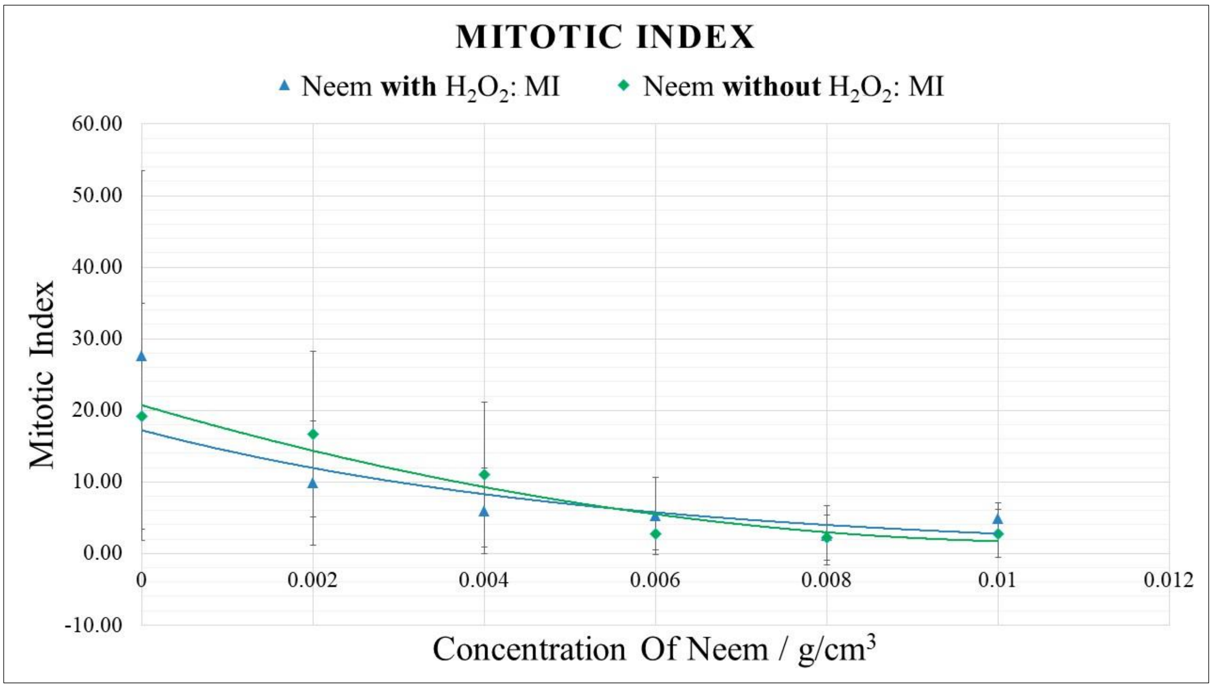
## 3.4 VISUAL REPRESENTATION USING GRAPHS



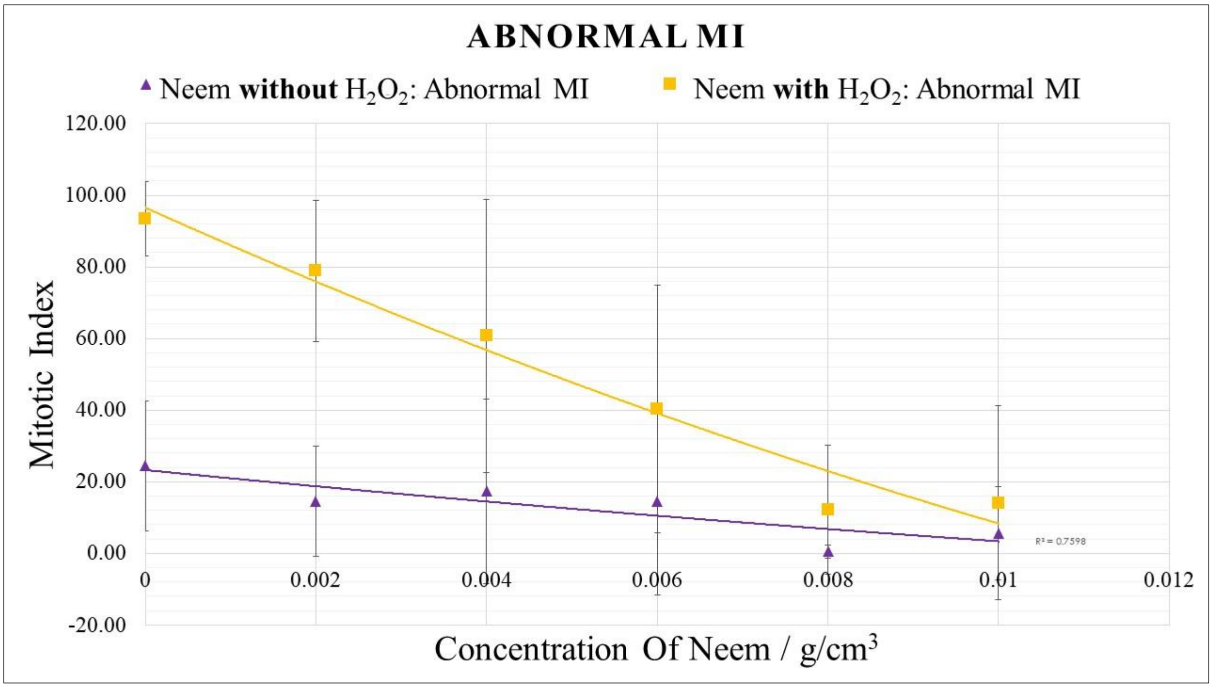

*Graph 1: MI and A-MI in set-up with neem without $H_2O_2$*




*Graph 2: MI and A-MI in set-up with neem with $H_2O_2$*




*Graph 3: Comparison of MI between set-up without and with $H_2O_2$*




*Graph 4: Comparison of A-MI between set-up without and with $H_2O_2$*

## 3.5 DATA ANALYSIS: STATISTICAL TESTS

ANOVA Test and Tukey test were used for this investigation.

### *3.5.1 MI in Neem **without** $H_2O_2$*

Type I error is avoided in ANOVA where all independent variables are compared together[30,31]. In ANOVA test, two hypothesis are made, the null and alternative hypothesis, tested at 0.05 significance level.

**Null hypothesis:** There is no statistically significant difference between increasing concentrations of neem in the set-up without $H_2O_2$ and MI.

**Alternative hypothesis:** There is a statistically significant difference between increasing concentrations of neem in the set-up without $H_2O_2$ and MI.

| Anova: Single Factor Neem w/o H2O2 MI | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| 0 | 10 | 191.28 | 19.128 | 248.5329 | | |
| 0.002 | 10 | 166.55 | 16.655 | 134.179 | | |
| 0.004 | 10 | 109.95 | 10.995 | 102.1179 | | |
| 0.006 | 10 | 27.43 | 2.743 | 4.834579 | | |
| 0.008 | 10 | 22.46 | 2.246 | 10.17132 | | |
| 0.010 | 10 | 27.97 | 2.797 | 11.44785 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 2883.462 | 5 | 576.6924 | 6.767585 | 5.8E-05 | 2.38607 |
| Within Groups | 4601.551 | 54 | 85.21391 | | | |
| | | | | | | |
| Total | 7485.013 | 59 | | | | |

Using Microsoft Excel's ANOVA test software, the above summary is obtained. Since the p-value (5.8E-05) is less than 0.05, there is sufficient evidence to suggest that one or more comparisons are statistically different. Hence the null hypothesis is rejected. However, ANOVA cannot tell which comparisons are statistically different. Hence the Tukey test was chosen based on its ability to look at 6 independent variables inclusive of the control, accounting for error etc[32].

Tukey test was done using astatsa.com. Table 9 shows the Q-Critical values used.

*Table 9: Tukey test Q-Critical value used*

| | | |
|---|---|---|
| Number of independent variables, k = 6<br>Degree of freedom, v = 54 | Significance level, α = 0.05 | 4.1782 |

If the Tukey HSD Q statistics is above the Q-critical values, there is a significant difference in the data at a confidence interval of 95%.

*Table 10: Tukey results (MI in set-up with neem without $H_2O_2$)*

| Comparisons | MI in the set-up with neem without $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | Inference |
| 0.000 vs 0.002 | 0.8472 | 0.8999947 | insignificant |
| 0.000 vs 0.004 | 2.7861 | 0.3736337 | insignificant |
| 0.000 vs 0.006 | 5.613 | 0.0028093 | ** p<0.05 |
| 0.000 vs 0.008 | 5.7832 | 0.001922 | ** p<0.05 |
| 0.000 vs 0.010 | 5.5945 | 0.0029256 | ** p<0.05 |
| 0.002 vs 0.004 | 1.9389 | 0.7180143 | insignificant |
| 0.002 vs 0.006 | 4.7658 | 0.0166407 | * p<0.05 |
| 0.002 vs 0.008 | 4.936 | 0.0118308 | * p<0.05 |
| 0.002 vs 0.010 | 4.7473 | 0.0172595 | * p<0.05 |
| 0.004 vs 0.006 | 2.8269 | 0.3570371 | insignificant |
| 0.004 vs 0.008 | 2.9971 | 0.2932046 | insignificant |
| 0.004 vs 0.010 | 2.8084 | 0.3645242 | insignificant |
| 0.006 vs 0.008 | 0.1703 | 0.8999947 | insignificant |
| 0.006 vs 0.010 | 0.0185 | 0.8999947 | insignificant |
| 0.008 vs 0.010 | 0.1888 | 0.8999947 | insignificant |

### 3.5.2 *A-MI in Neem **without** $H_2O_2$*

**Null hypothesis:** There is no statistical difference between increasing concentrations of neem in the set-up without $H_2O_2$ and A-MI.

**Alternative hypothesis:** There is statistical difference between increasing concentrations of neem in the set-up without $H_2O_2$ and A-MI.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 3654.975 | 5 | 730.995 | 2.095412 | 0.080053 | 2.38607 |
| Within Groups | 18838.17 | 54 | 348.855 | | | |
| | | | | | | |
| Total | 22493.15 | 59 | | | | |

Null Hypothesis accepted. Hence no Tukey test was done.

### 3.5.3 *MI in Neem **with** $H_2O_2$*

**Null hypothesis:** There is no statistical difference between increasing concentrations of neem in the set-up with $H_2O_2$ and MI.

**Alternative hypothesis:** There is statistical difference between increasing concentrations of neem in the set-up with $H_2O_2$ and MI.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 4289.262 | 5 | 857.8524 | 6.073286 | 0.000157 | 2.38607 |
| Within Groups | 7627.506 | 54 | 141.2501 | | | |
| | | | | | | |
| Total | 11916.77 | 59 | | | | |

Null hypothesis was rejected. Tukey test was done. Q critical values are in Table 9.

*Table 11: Tukey results (MI in the set-up with neem with $H_2O_2$)*

| Comparisons | MI in the set up with neem with $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | |
| 0.000 vs 0.002 | 4.7375 | 0.0175975 | * p<0.05 |
| 0.000 vs 0.004 | 5.7592 | 0.0020292 | ** p<0.05 |
| 0.000 vs 0.006 | 5.9548 | 0.0013008 | ** p<0.05 |
| 0.000 vs 0.008 | 6.6828 | 0.0010053 | ** p<0.05 |
| 0.000 vs 0.010 | 6.0442 | 0.0010594 | ** p<0.05 |
| 0.002 vs 0.004 | 1.0217 | 0.8999947 | insignificant |
| 0.002 vs 0.006 | 1.2173 | 0.8999947 | insignificant |
| 0.002 vs 0.008 | 1.9453 | 0.7154867 | insignificant |
| 0.002 vs 0.010 | 1.3067 | 0.8999947 | insignificant |
| 0.004 vs 0.006 | 0.1956 | 0.8999947 | insignificant |
| 0.004 vs 0.008 | 0.9235 | 0.8999947 | insignificant |
| 0.004 vs 0.010 | 0.285 | 0.8999947 | insignificant |
| 0.006 vs 0.008 | 0.728 | 0.8999947 | insignificant |
| 0.006 vs 0.010 | 0.0894 | 0.8999947 | insignificant |
| 0.008 vs 0.010 | 0.6386 | 0.8999947 | insignificant |

### *3.5.4 A-MI in Neem **with** $H_2O_2$*

**Null hypothesis:** There is no statistical difference between increasing concentrations of neem in the set-up with $H_2O_2$ and A-MI.

**Alternative hypothesis:** There is statistical difference between increasing concentrations of neem in the set-up with $H_2O_2$ and A-MI.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 56534.03 | 5 | 11306.81 | 16.16185 | 1.02E-09 | 2.38607 |
| Within Groups | 37778.32 | 54 | 699.5985 | | | |
| | | | | | | |
| Total | 94312.34 | 59 | | | | |

Null Hypothesis was rejected. Tukey test was done. Q critical values are in Table 9.

*Table 12: Tukey Test (A-MI in the set-up with neem with $H_2O_2$)*

| Comparisons | A-MI in the set up with neem with $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | |
| 0.000 vs 0.002 | 1.7277 | 0.8020583 | insignificant |
| 0.000 vs 0.004 | 3.9052 | 0.0799202 | insignificant |
| 0.000 vs 0.006 | 6.3432 | 0.0010053 | ** $p<0.05$ |
| 0.000 vs 0.008 | 9.7123 | 0.0010053 | ** $p<0.05$ |
| 0.000 vs 0.010 | 9.4858 | 0.0010053 | ** $p<0.05$ |
| 0.002 vs 0.004 | 2.1775 | 0.6230857 | insignificant |
| 0.002 vs 0.006 | 4.6155 | 0.0223118 | * $p<0.05$ |
| 0.002 vs 0.008 | 7.9846 | 0.0010053 | ** $p<0.05$ |
| 0.002 vs 0.010 | 7.7581 | 0.0010053 | ** $p<0.05$ |
| 0.004 vs 0.006 | 2.438 | 0.5194205 | insignificant |
| 0.004 vs 0.008 | 5.8071 | 0.0018221 | ** $p<0.05$ |
| 0.004 vs 0.010 | 5.5806 | 0.0030187 | ** $p<0.05$ |
| 0.006 vs 0.008 | 3.3691 | 0.1806194 | insignificant |
| 0.006 vs 0.010 | 3.1426 | 0.2446772 | insignificant |
| 0.008 vs 0.010 | 0.2266 | 0.8999947 | insignificant |

# 4 DISCUSSION

**In the absence of $H_2O_2$, as the concentration of neem increases, both the MI and A-MI falls(graph 1).**

As the neem concentration increases, content of nimbolide and anti-proliferative phytochemicals increases. Hence, the root cells undergo a slower rate of proliferation and MI falls. At a 95% confidence interval, the difference between the MI of the control and concentrations 0.006g/cm$^3$ and above and the difference between the MI in 0.002g/cm$^3$ and concentrations 0.006g/cm$^3$ and above is significant(Table 10). Therefore, neem concentration of 0.006g/cm$^3$ gives rise to significant anti-cell proliferation effect. After the concentration of 0.006g/cm$^3$, there is saturation of these phytochemicals in the cell and therefore anything higher than 0.006g/cm$^3$ does not make a significant difference.

Researches[3] suggests neem causes mutagenic effects above 0.010 g/cm$^3$. Since the range used in this investigation was below 0.01g/cm$^3$, no adverse effects was seen in terms of A-MI, 0.01g/cm$^3$ of neem can be used.

**In the absence of $H_2O_2$, there is no significant difference in the fall of A-MI as concentration of neem increases (Section 3.5.2).** Some researches[3] state that neem cause abnormalities in mitosis while other researches suggest that phytochemicals in neem like nimbolide may help reduce cytotoxicity and thereby protecting the DNA[21]. Hence at low concentrations of neem, both toxic and anti-genotoxic effects of neem may take place. There could also be inherent abnormalities in the onion roots. Hence, results obtained is net effect.

**In the presence of $H_2O_2$, as the concentration of neem increases, both the MI and A-MI fall(graph 2).** As neem concentration increases, A-MI falls because disruption of KEAP1-Nrf2 complex increases releasing more Nrf2 protein which helps to preventing oxidative stress on the DNA undergoing duplication and division. Increasing neem concentration, increases concentration of quercetin and rutin which get oxidized in place of the DNA. This can explain the frothing seen when neem and $H_2O_2$ are mixed, helping to prevent oxidative stress on the DNA (Figure 5). However, throughout these processes, the phytochemical could get metabolized and fewer of these phytochemicals are left to lower MI.

As the concentration increases in the set-up with $H_2O_2$, the difference between the MI and A-MI fall(Graph 2). This could be because the number cells undergoing abnormal mitosis falls more than the number of cells undergoing mitosis(abnormal and normal).

At a 95% confidence interval, significant differences only occur for the MI between the control and 0.002g/cm$^3$ concentration (Table 11) and the A-MI between control and concentrations of 0.006g/cm$^3$ and above of neem (Table 12). No other comparisons are significantly different. Graph 2 also shows that the error bars only start to overlap at 0.006g/cm$^3$. Hence a minimum neem concentration of 0.006g/cm$^3$ has to be used to reduce both MI and A-MI significantly.

**As the concentration of neem increases, the MI in the set-up with $H_2O_2$ decreases to a less extent compared to the MI in set-up without $H_2O_2$ (Graph 3).** In the set-up with $H_2O_2$, nimbolide has to reduce oxidative stress and only remaining nimbolide will reduce cell proliferation. However, in the set-up without $H_2O_2$ all the nimbolide is being used to reduce cell proliferation. Therefore, the set up with $H_2O_2$ and neem will experience a smaller magnitude in the fall of the MI compared to the set-up with only neem.

Graph 3 also shows that MI and A-MI start to plateau at higher concentrations including and above 0.008g/cm$^3$. This could be due to the saturation of phytochemicals and the definite amount of proliferation pathways occurring. Hence any increase in the concentration of phytochemicals do not cause any significant difference in the MI.

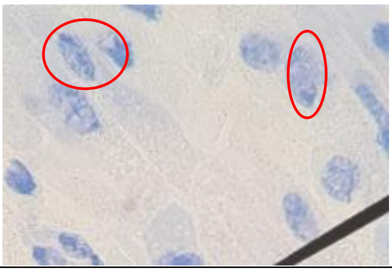
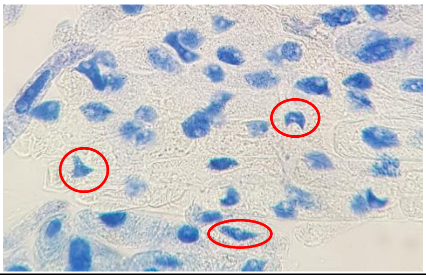
**As the concentration of neem increases, A-MI for the set-up with $H_2O_2$ decreases with a larger extent compared to the A-MI in set-up without $H_2O_2$(Graph 4).** In the set-up with $H_2O_2$, high levels of oxidative damage occurs to the DNA than in the set-up without $H_2O_2$. The difference in the A-MI between the set-ups with and without $H_2O_2$ is large and reduce as concentration increases. Moreover, error bars do not overlap until 0.004g/cm$^3$. This suggests that for the effects of $H_2O_2$ to be reduced to give the same results as the set-up without $H_2O_2$ a concentration of 0.04g/cm$^3$ is required.

**To add on, the A-MI is higher than the MI in both set-ups without or with $H_2O_2$.** This can be attributed to the formula used to calculate the A-MI because the number of cells undergoing mitosis is much smaller than the number of cells present. Hence the A-MI calculated would be generally higher due to the denominator being significantly lower in magnitude than that of the MI.

Overall, the results support the hypothesis made.

## 5 EVALUATION AND EXTENSIONS

Abnormal and normal mitotic index, used to detect DNA damage may not be all-encompassing. Other types of abnormal figures were seen not classified under the mitotic index. Below shows some example:

| Type of abnormality | Self-taken Photos |
|---|---|
| Apoptosis: Complete disintegration of the nucleus and the cell |  |
| Fragmentation of the nucleus, nucleus is not concentrated around one area rather is spread out unevenly. |  |
| Abnormally shaped nucleus having pointed edges |  |

None of the above nuclei are undergoing mitosis but reflect DNA damage. A preliminary trial was done for this extension (Section 9). In the presence of $H_2O_2$, as the neem concentration increases, the number of abnormalities decreases but no trend was observed in the absence of $H_2O_2$. Therefore, further investigation in this area can be done.

Another limitation is that the duration was kept at 24h. However, the effects of neem could possibly remain after 24h. A trial was done to investigate what the effects were after 24h at the 48h mark(Section 10). Although some concentrations showed effects, the data predominantly showed that neem could not alter the mitotic index at the 48h mark. Hence, an investigation can be conducted by creating more intervals for testing between 24h and 48h and testing MI and A-MI.

Additionally, this experiment investigated one type of ROS. An extension could be done to see whether neem can fight against other forms of radicals like Cl•.

Moreover, there is biological variation of the onions used. Hence, it is nearly impossible to maintain the exact same health level, root length, stage of growth etc. of every single onion. Hence this increases the variation in mitotic index obtained.

Furthermore, the investigation is based on the Allium cepa working as a model of cancer cells. However, Allium cepa is not a true model because it does not fully reflect the human body and its mechanisms. Hence, conclusions obtained in this investigation cannot be fully applied to other species, like humans.

Despite these limitations the experiment has strengths. Using *Allium cepa* as a model has the least safety and ethical issue as opposed to using other mediums like actual human and/or animal tissue. Additionally, this experiment can be reproduced easily as the cost, and duration of this experiment is minimal. Moreover, results obtained have significant differences between independent variables and this is a strength of the investigation.

# 6 CONCLUSION

In conclusion, as the concentration of neem increases without $H_2O_2$ in the mixture, both the MI and A-MI reduces. This trend was attributed to the properties of the phytochemicals present in neem (e.g. nimbolide) which disrupt cell proliferation pathways and disrupts cell regulatory proteins like CDKs and cyclin proteins which causes cell cycle arrest.

Additionally, in the set-up with $H_2O_2$ as the concentration of neem increases, both MI and A-MI falls. This was attributed to the increasing flavonoids present in neem (as concentration of neem increases) which gets oxidised in place of DNA molecules which allow the increasing normal progression of mitosis. However, at the same time, nimbolide works to disrupt cell proliferation and slows down mitosis. Hence both MI and A-MI falls as concentration increases.

# 7 BIBLIOGRAPHY

1. Susan Scutti. Cancer surpasses heart disease as leading cause of death in many US countries. CNN. 2018 Nov 12 [accessed 2019 Apr 4]. https://edition.cnn.com/2018/11/12/health/cancer-surpasses-heart-disease-study/index.html

2. Side Effects of Cancer Treatment - National Cancer Institute. 2018 [accessed 2019 Apr 4]. https://www.cancer.gov/about-cancer/treatment/side-effects

3. Adegbite AE, Ayodele MS, Odunbaku KR, Idehen EO. Mutagenic effect of neem leaf extract used in traditional medicine on Allium cepa (L.). Scientific Research and Essays. 2009 [accessed 2018 Oct 13];4(11):1315–1321. http://www.academicjournals.org/SRE

4. Spencer JPE, Jenner A, Chimel K, Aruoma OI, Cross CE, Wu R, Halliwell B. DNA strand breakage and base modification induced by hydrogen peroxide treatment of human respiratory tract epithelial cells. 1995. https://core.ac.uk/download/pdf/81158349.pdf

5. Lim S, Kaldis P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. The Company of Biologists Ltd. 2013;140(15):3079–3093. doi:10.1242/dev.091744

6. Khan Academy. Cell cycle regulators. [accessed 2019 Apr 4]. https://www.khanacademy.org/science/biology/cellular-molecular-biology/stem-cells-and-cancer/a/cell-cycle-regulators

7. Campbell NA, B.Reece J, A.Urry L, L.Cain M, A.Wasserman S, V.Minorsky P, B.Jackson R. Biology: A global Approach. 10th ed. Pearson; 2015.

8. What Is Cancer? - National Cancer Institute. 2015 [accessed 2019 Jun 11]. https://www.cancer.gov/about-cancer/understanding/what-is-cancer

9. Ingrid Lobo. Chromosome Abnormalities and Cancer Cytogenetics. 2008 [accessed 2019 Jun 11]. https://www.nature.com/scitable/topicpage/chromosome-abnormalities-and-cancer-cytogenetics-879. doi:10.1038/ng1215

10. Allium IN, Cells CR. Mitotic Aberrations Induced By Sunflower Seed Oil. 1962.

11. Bavle RM. Nuclear vacuolization: Giant Lochkern-like cells. Journal of oral and maxillofacial pathology : JOMFP. 2016 [accessed 2019 Jun 13];20(3):339–341. http://www.ncbi.nlm.nih.gov/pubmed/27721594. doi:10.4103/0973-029X.190895

12. Aravinthan A, Verma S, Coleman N, Davies S, Allison M, Alexander G. Vacuolation in hepatocyte nuclei is a marker of senescence. Journal of Clinical Pathology. 2012;65(6):557–560. doi:10.1136/jclinpath-2011-200641

13. Ahmida R, Elshatshat S. Genotoxic evaluations of Allium cepa L. using different concentrations of synthesized butenolide. Pelagia Research Library Asian Journal of Plant Science and Research. [accessed 2019 Jun 13];2013(4):8–12. www.pelagiaresearchlibrary.com

14. Leslie Redpath J, Bengtsson U, DeSimone J, Lao X, Wang X, Stanbridge EJ. Sticky anaphase aberrations after G2-phase arrest of gamma-irradiated human skin fibroblasts: TP53 independence of formation and TP53 dependence of consequences. Radiation research. 2003 [accessed 2019 Jun 13];159(1):57–71. http://www.ncbi.nlm.nih.gov/pubmed/12492369

15. Apel K, Hirt H. REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction. Annu. Rev. Plant Biol. 2004 [accessed 2018 Jul 6];55:373–99. http://www.heribert-hirt.info/pdf/prr85_ros.pdf. doi:10.1146/annurev.arplant.55.031903.141701

16. Dong J, Liu B, Zhu R. Targeting ROS for Cancer Therapy. Chemotherapy. 2016 [accessed 2018 Jul 6];05(02):1–7. http://www.omicsgroup.org/journals/targeting-ros-for-cancer-therapy-2167-7700-1000199.php?aid=73644. doi:10.4172/2167-7700.1000199

17. López-Lázaro M. Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy. Cancer letters. 2007 [accessed 2018 May 6];252(1):1–8. http://www.ncbi.nlm.nih.gov/pubmed/17150302. doi:10.1016/j.canlet.2006.10.029

18. McCain J. The MAPK (ERK) Pathway: Investigational Combinations for the Treatment Of BRAF-Mutated Metastatic Melanoma. P & T : a peer-reviewed journal for formulary

management. 2013 [accessed 2019 Apr 4];38(2):96–108. http://www.ncbi.nlm.nih.gov/pubmed/23599677

19. Rueff J, Brás A, Cristóvão L, Mexia J, Sáda Costa M, Pires V. DNA strand breaks and chromosomal aberrations induced by H2O2and60Co γ-radiation. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis. 1993;289(2):197–204. doi:10.1016/0027-5107(93)90070-V

20. R. Subapriya, S. Nagini. Medicinal Properties of Neem Leaves: A Review. 2005 [accessed 2018 Jul 7]. http://medicalmate.gr/img/cms/Vivliothiki Gia Epaggelmaties Ygeias/wound management/Allegato NE 01.pdf

21. Hao F, Kumar S, Yadav N, Chandra D. Neem components as potential agents for cancer prevention and treatment. Biochimica et biophysica acta. 2014 [accessed 2018 Jul 7];1846(1):247–57. http://www.ncbi.nlm.nih.gov/pubmed/25016141. doi:10.1016/j.bbcan.2014.07.002

22. Cotelle N. Role of flavonoids in oxidative stress. Curr Top Med Chem. 2001;1(6):569–590.

23. Kuwana T. The Role of Mitochondrial Outer Membrane Permeabilization (MOMP) in Apoptosis: Studying Bax Pores by Cryo-Electron Microscopy. 1st ed. Elsevier Inc.; 2018. doi:10.1016/bs.abl.2017.12.002

24. Wang L, Phan DDK, Zhang J, Ong P-S, Thuya WL, Soo R, Wong AL-A, Yong WP, Lee SC, Ho PC-L, et al. Anticancer properties of nimbolide and pharmacokinetic considerations to accelerate its development. Oncotarget. 2016 [accessed 2019 Feb 27];7(28):44790–44802. http://www.ncbi.nlm.nih.gov/pubmed/27027349. doi:10.18632/oncotarget.8316

25. Ma Q. Role of nrf2 in oxidative stress and toxicity. Annual review of pharmacology and toxicology. 2013 [accessed 2019 Apr 5];53:401–26. http://www.ncbi.nlm.nih.gov/pubmed/23294312. doi:10.1146/annurev-pharmtox-011112-140320

26. Deshmukh P, Unni S, Krishnappa G, Padmanabhan B. The Keap1-Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. Biophysical reviews. 2017 [accessed 2019 Mar 9];9(1):41–56. http://www.ncbi.nlm.nih.gov/pubmed/28510041. doi:10.1007/s12551-016-0244-4

27. LabChem. Hydrogen Peroxide, 3% w/w. 2017. www.labchem.com

28. Pubchem. Hydrogen Peroxide. Pubchem. [accessed 2018 May 21]. https://pubchem.ncbi.nlm.nih.gov/compound/784#section=Top

29. ThermoFisher Scientific. Safety Data Sheet: Toluidine Blue O. 2010. https://www.fishersci.com/store/msds?partNumber=T16125&productDescription=TOLUIDINE+BLUE+O+CERT+BIO+25G&vendorId=VN00033897&countryCode=US&language=en

30. University of California B. Type I and Type II errors. https://www.stat.berkeley.edu/~hhuang/STAT141/Lecture-FDR.pdf

31. What are type I and type II errors? minitab. 2019 [accessed 2019 Mar 9]. https://support.minitab.com/en-us/minitab/18/help-and-how-to/statistics/basic-statistics/supporting-topics/basics/type-i-and-type-ii-error/

32. IBM Knowledge Center. One-Way ANOVA Post Hoc Tests. IBM Knowledge Center. [accessed 2019 Mar 9]. https://www.ibm.com/support/knowledgecenter/en/SSLVMB_23.0.0/spss/base/idh_onew_post.html

33. Ronald Ross Watson, Victor R. Preedy. Botanical Medicine in Clinical Practice - Google Books. 2008 [accessed 2019 Feb 27]. https://books.google.com.sg/books?hl=en&lr=&id=j5PdVtMgVLYC&oi=fnd&pg=PA85&dq=neem+extract+and+anti+cancer+mitotic+index+root+tip&ots=n95Fs4J7E_&sig=Ft1XkJc0xvu_9smsg2hFYyxzJCo#v=onepage&q&f=false

34. Elmore S. Apoptosis: a review of programmed cell death. Toxicologic pathology. 2007

[accessed 2019 Mar 9];35(4):495–516. http://www.ncbi.nlm.nih.gov/pubmed/17562483. doi:10.1080/01926230701320337

# 8 Appendix

## 8.1 Background information

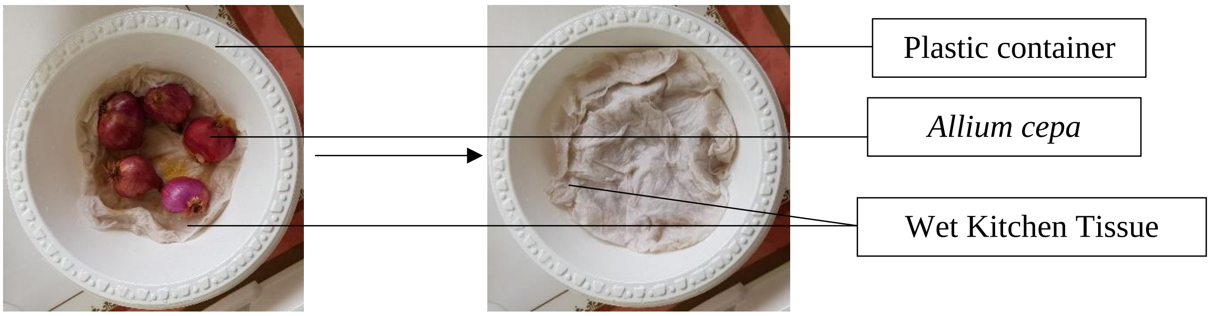
*Appendix Table 1: Scientific classification of neem*

| Scientific classification[33] | |
|---|---|
| Kingdom | Plantae |
| Division | Rutales |
| Order | Sapindales |
| Family | Meliaceae |
| Genus | Azadirachta |
| Species | Indica |

## 8.2 Methodology

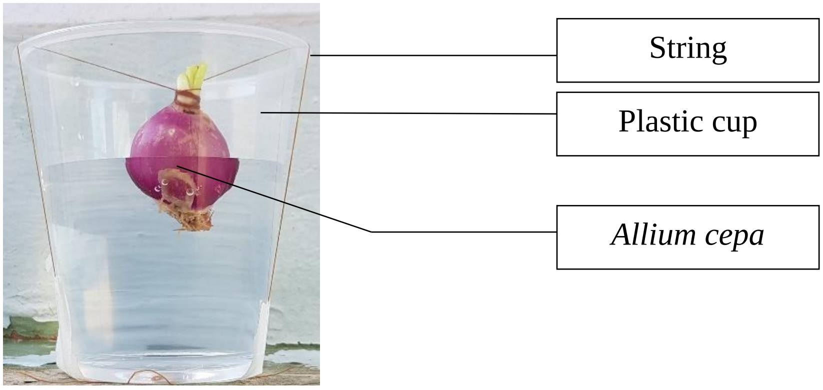
### 8.2.1 Protocol for growing onions

1. Soak the onion in a tub of water for 1h.
2. Place the soaked onions on top of the wet kitchen tissue placed in a plastic container, evenly spaced out.
3. Place another wet kitchen tissue, about 2 cm in depth, on top of the onions. Make sure that are no "gaps" in which air can easily go in and out.




*Appendix Figure 1:Step 2 of protocol ; Appendix Figure 2: Step 3 of protocol*

4. Place the plastic container with onions outdoors for 1 day.
5. Transfer the onions into the plastic cups. Hold up the onions using strings. Leave them outside on the balcony until the roots are about 1 inch long.




*Appendix Figure 3: Setup of Onion*

### 8.2.2 *Other steps and protocols with pictures:*

1. Using a stone mortar and pestle, grind the neem leaves using a stone mortar and pestle.


*Appendix Figure 4: Stone and mortar pestle*

## 8.3 RAW DATA FOR THE INVESTIGATION

*Appendix Table 2: 0.000g/cm$^3$ Neem without $H_2O_2$*

| Concentration | Replicate | Root no. | Prophase | Metaphase | Anaphase | Telophase | A-Prophase | A-metaphase | A-Anaphase | A-telophase | Total number of cells in normal mitosis | Total number of cells in Abnormal Mitosis | Total Number of cells | MI-N | MI-A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 5 | 3 | 239 | 3.35 | 37.50 |
| | | 2 | 28 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 29 | 2 | 313 | 9.90 | 6.45 |
| | 1 | Average | 16 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 17 | 3 | 276 | 6.63 | 21.98 |
| | | 1 | 24 | 0 | 0 | 2 | 0 | 6 | 0 | 0 | 26 | 6 | 240 | 13.33 | 18.75 |
| | | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 2 | 54 | 7.41 | 50.00 |
| | 2 | Average | 13 | 0 | 0 | 1 | 1 | 3 | 0 | 1 | 14 | 4 | 147 | 10.37 | 34.38 |
| | | 1 | 6 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 8 | 1 | 46 | 19.57 | 11.11 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 92 | 3.26 | 66.67 |
| | 3 | Average | 4 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 5 | 2 | 69 | 11.41 | 38.89 |
| | | 1 | 3 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 3 | 5 | 143 | 5.59 | 62.50 |
| | | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 77 | 5.19 | 50.00 |
| | 4 | Average | 3 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 3 | 4 | 110 | 5.39 | 56.25 |
| | | 1 | 127 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 128 | 1 | 205 | 62.93 | 0.78 |
| | | 2 | 75 | 0 | 0 | 0 | 3 | 1 | 1 | 0 | 75 | 5 | 152 | 52.63 | 6.25 |
| | 5 | Average | 101 | 1 | 0 | 0 | 2 | 1 | 1 | 0 | 102 | 3 | 179 | 57.78 | 3.51 |
| | | 1 | 12 | 1 | 0 | 0 | 4 | 1 | 0 | 0 | 13 | 5 | 91 | 19.78 | 27.78 |
| | | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 3 | 113 | 4.42 | 60.00 |
| | 6 | Average | 7 | 1 | 0 | 0 | 2 | 1 | 0 | 1 | 8 | 4 | 102 | 12.10 | 43.89 |
| | | 1 | 53 | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 54 | 5 | 143 | 41.26 | 8.47 |
| | | 2 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 120 | 25.83 | 0.00 |
| | 7 | Average | 42 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 43 | 3 | 132 | 33.55 | 4.24 |
| | | 1 | 27 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 27 | 2 | 175 | 16.57 | 6.90 |
| | | 2 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 16 | 3 | 93 | 20.43 | 15.79 |
| | 8 | Average | 22 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 22 | 3 | 134 | 18.50 | 11.34 |
| | | 1 | 33 | 1 | 2 | 0 | 4 | 0 | 0 | 0 | 36 | 4 | 124 | 32.26 | 10.00 |
| | | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 111 | 2.70 | 33.33 |
| | 9 | Average | 18 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 19 | 3 | 118 | 17.48 | 21.67 |
| | | 1 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 15 | 2 | 128 | 13.28 | 11.76 |
| | | 2 | 14 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 15 | 1 | 70 | 22.86 | 6.25 |
| | 10 | Average | 15 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 15 | 2 | 99 | 18.07 | 9.01 |
| | Average | | 24 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 25 | 3 | 136 | 19.13 | 24.51 |
| 0.000 | SD | | 29 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 29 | 1 | 57 | 15.76 | 18.16 |

*Appendix Table 3: 0.002g/cm$^3$ Neem without $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 61 | 3 | 0 | 0 | 0 | 10 | 0 | 0 | 64 | 10 | 517 | 14.31 | 13.51 |
| | | 2 | 82 | 0 | 0 | 0 | 10 | 11 | 0 | 0 | 82 | 21 | 562 | 18.33 | 20.39 |
| | 1 | Average | 72 | 2 | 0 | 0 | 5 | 11 | 0 | 0 | 73 | 16 | 540 | 16.32 | 16.95 |
| | | 1 | 9 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 9 | 4 | 128 | 10.16 | 30.77 |
| | | 2 | 19 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 19 | 2 | 218 | 9.63 | 9.52 |
| | 2 | Average | 14 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 14 | 3 | 173 | 9.89 | 20.15 |
| | | 1 | 6 | 0 | 0 | 0 | 6 | 4 | 0 | 0 | 6 | 10 | 100 | 16.00 | 62.50 |
| | | 2 | 15 | 1 | 0 | 0 | 3 | 1 | 0 | 0 | 16 | 4 | 174 | 11.49 | 20.00 |
| | 3 | Average | 11 | 1 | 0 | 0 | 5 | 3 | 0 | 0 | 11 | 7 | 137 | 13.75 | 41.25 |
| | | 1 | 7 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 7 | 3 | 86 | 11.63 | 30.00 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 218 | 0.92 | 50.00 |
| | 4 | Average | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 4 | 2 | 152 | 6.27 | 40.00 |
| | | 1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 1 | 111 | 8.11 | 11.11 |
| | | 2 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 10 | 1 | 186 | 5.91 | 9.09 |
| | 5 | Average | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 9 | 1 | 149 | 7.01 | 10.10 |
| | | 1 | 12 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 12 | 1 | 128 | 10.16 | 7.69 |
| | | 2 | 78 | 4 | 0 | 0 | 11 | 0 | 0 | 0 | 82 | 11 | 222 | 41.89 | 11.83 |
| | 6 | Average | 45 | 2 | 0 | 0 | 6 | 0 | 0 | 0 | 47 | 6 | 175 | 26.02 | 9.76 |
| | | 1 | 128 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 135 | 0 | 200 | 67.50 | 0.00 |
| | | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 236 | 2.54 | 0.00 |
| | 7 | Average | 67 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 71 | 0 | 218 | 35.02 | 0.00 |
| | | 1 | 5 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 6 | 1 | 105 | 6.67 | 14.29 |
| | | 2 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 137 | 5.11 | 0.00 |
| | 8 | Average | 6 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 7 | 1 | 121 | 5.89 | 7.14 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 0.00 | 0.00 |
| | | 2 | 59 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 64 | 0 | 89 | 71.91 | 0.00 |
| | 9 | Average | 30 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 77 | 35.96 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 0.00 | 0.00 |
| | | 2 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 144 | 20.83 | 0.00 |
| | 10 | Average | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 95 | 10.42 | 0.00 |
| | Average | | 27 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 28 | 4 | 184 | 16.66 | 14.54 |
| 0.002 | SD | | 25 | 1 | 0 | 0 | 2 | 3 | 0 | 0 | 26 | 5 | 131 | 11.58 | 15.39 |

*Appendix Table 4: 0.004 g/cm$^3$ Neem without $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 46 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 46 | 7.0 | 137 | 38.69 | 13.21 |
| | | 2 | 12 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 15 | 0.0 | 132 | 11.36 | 0.00 |
| | 1 | Average | 29 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 31 | 4 | 135 | 25.02 | 6.60 |
| | | 1 | 8 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 8 | 3 | 89 | 12.36 | 27.27 |
| | | 2 | 10 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 68 | 16.18 | 0.00 |
| | 2 | Average | 9 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 10 | 2 | 79 | 14.27 | 13.64 |
| | | 1 | 7 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 8 | 0 | 77 | 10.39 | 0.00 |
| | | 2 | 12 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 111 | 12.61 | 0.00 |
| | 3 | Average | 10 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 11 | 0 | 94 | 11.50 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 86 | 3.49 | 100.00 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 80 | 1.25 | 0.00 |
| | 4 | Average | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 2 | 83 | 2.37 | 50.00 |
| | | 1 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 0 | 94 | 50.00 | 0.00 |
| | | 2 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 164 | 4.88 | 0.00 |
| | 5 | Average | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 129 | 27.44 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 70 | 1.43 | 100.00 |
| | | 2 | 3 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 3 | 3 | 330 | 1.82 | 50.00 |
| | 6 | Average | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 2 | 2 | 200 | 1.62 | 75.00 |
| | | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 175 | 2.29 | 50.00 |
| | | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 249 | 2.41 | 0.00 |
| | 7 | Average | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 1 | 212 | 2.35 | 25.00 |
| | | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 104 | 1.92 | 0.00 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 1 | 0 | 116 | 0.86 | 0.00 |
| | 8 | Average | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 110 | 1.39 | 0.00 |
| | | 1 | 42 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 42 | 3 | 116 | 38.79 | 6.67 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 0.00 | 0.00 |
| | 9 | Average | 42 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 21 | 2 | 98 | 19.40 | 3.33 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 128 | 0.00 | 0.00 |
| | | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 98 | 9.18 | 0.00 |
| | 10 | Average | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 113 | 4.59 | 0.00 |
| | Average | | 13 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 11 | 1 | 125 | 11.00 | 17.36 |
| 0.004 | SD | | 15 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 11 | 1 | 46 | 10.11 | 25.77 |

## *Appendix Table 5: 0.006 g/cm$^3$ Neem without $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1.0 | 555 | 0.18 | 100.00 |
| | | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1.0 | 500 | 0.40 | 50.00 |
| | 1 | Average | 0.5 | 0 | 0 | 0 | 0.5 | 0 | 0.5 | 0 | 0.5 | 1.0 | 527.5 | 0.29 | 75.00 |
| | | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 37 | 2.70 | 0.00 |
| | | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0.0 | 47 | 8.51 | 0.00 |
| | 2 | Average | 2.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 | 0.0 | 42 | 5.61 | 0.00 |
| | | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0.0 | 167 | 1.80 | 0.00 |
| | | 2 | 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 5 | 1.0 | 118 | 5.08 | 16.67 |
| | 3 | Average | 4 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 4 | 0.5 | 142.5 | 3.44 | 8.33 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 46 | 0.00 | 0.00 |
| | | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | | 3 | 0.0 | 35 | 8.57 | 0.00 |
| | 4 | Average | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.5 | 0.0 | 40.5 | 4.29 | 0.00 |
| | | 1 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 1.0 | 145 | 2.76 | 25.00 |
| | | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0.0 | 57 | 8.77 | 0.00 |
| | 5 | Average | 4 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 4 | 0.5 | 101 | 5.77 | 12.50 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 73 | 0.00 | 0.00 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 84 | 1.19 | 0.00 |
| | 6 | Average | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.0 | 78.5 | 0.60 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 56 | 0.00 | 0.00 |
| | | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0.0 | 126 | 2.38 | 0.00 |
| | 7 | Average | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.5 | 0.0 | 91 | 1.19 | 0.00 |
| | | 1 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0.0 | 100 | 7.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1.0 | 69 | 1.45 | 100.00 |
| | 8 | Average | 3.5 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 3.5 | 0.5 | 84.5 | 4.22 | 50.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 119 | 0.00 | 0.00 |
| | | 2 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0.0 | 99 | 4.04 | 0.00 |
| | 9 | Average | 0.5 | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.0 | 109 | 2.02 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 112 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 95 | 0.00 | 0.00 |
| | 10 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 103.5 | 0.00 | 0.00 |
| | Average | | 1.85 | 0.15 | 0.00 | 0.00 | 0.15 | 0.00 | 0.10 | 0.00 | 2.00 | 0.3 | 132.00 | 2.74 | 14.58 |
| 0.006 | SD | | 1.55 | 0.47 | 0.00 | 0.00 | 0.24 | 0.00 | 0.21 | 0.00 | 1.47 | 0.4 | 142.25 | 2.20 | 26.30 |

## *Appendix Table 6: 0.008 g/cm$^3$ Neem without $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 446.00 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 235.00 | 0.00 | 0.00 |
| | 1 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 340.50 | 0.00 | 0.00 |
| | | 1 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0.0 | 65.00 | 9.23 | 0.00 |
| | | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0.0 | 162.00 | 1.85 | 0.00 |
| | 2 | Average | 4.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.5 | 0.0 | 113.50 | 5.54 | 0.00 |
| | | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 130.00 | 0.77 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 67.00 | 0.00 | 0.00 |
| | 3 | Average | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.0 | 98.50 | 0.38 | 0.00 |
| | | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0.0 | 107.00 | 3.74 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 64.00 | 0.00 | 0.00 |
| | 4 | Average | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.0 | 85.50 | 1.87 | 0.00 |
| | | 1 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0.0 | 180.00 | 7.22 | 0.00 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 99.00 | 1.01 | 0.00 |
| | 5 | Average | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0.0 | 139.50 | 4.12 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 94.00 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 74.00 | 0.00 | 0.00 |
| | 6 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 84.00 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 150.00 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 156.00 | 0.00 | 0.00 |
| | 7 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 153.00 | 0.00 | 0.00 |
| | | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.0 | 92.00 | 2.17 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 69.00 | 0.00 | 0.00 |
| | 8 | Average | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 80.50 | 1.09 | 0.00 |
| | | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.0 | 104.00 | 1.92 | 0.00 |
| | | 2 | 23 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 23 | 3.0 | 153.00 | 16.99 | 11.54 |
| | 9 | Average | 12.5 | 0 | 0 | 0 | 1 | 0.5 | 0 | 0 | 12.5 | 1.5 | 128.50 | 9.46 | 5.77 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 122.00 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 118.00 | 0.00 | 0.00 |
| | 10 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 120.00 | 0.00 | 0.00 |
| | Average | | 2.75 | 0.00 | 0.00 | 0.00 | 0.10 | 0.05 | 0.00 | 0.00 | 2.75 | 0.2 | 134.35 | 2.25 | 0.58 |
| 0.008 | SD | | 4.15 | 0.00 | 0.00 | 0.00 | 0.32 | 0.16 | 0.00 | 0.00 | 4.15 | 0.5 | 76.49 | 3.19 | 1.82 |

## *Appendix Table 7: 0.010 g/cm$^3$ Neem without $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 3.0 | 427 | 1.41 | 50.00 |
| | | 2 | 14 | 0 | 0 | 1 | 1 | 0 | 0 | 5 | 15 | 6.0 | 111 | 18.92 | 28.57 |
| | 1 | Average | 8.5 | 0 | 0 | 0.5 | 0.5 | 0 | 1.5 | 2.5 | 9 | 4.5 | 269 | 10.16 | 39.29 |
| | | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 159 | 0.63 | 0.00 |
| | | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 1.0 | 61 | 4.92 | 33.33 |
| | 2 | Average | 1.5 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 1.5 | 0.5 | 110 | 2.77 | 16.67 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 76 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 86 | 0.00 | 0.00 |
| | 3 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 81 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 39 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 33 | 0.00 | 0.00 |
| | 4 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 36 | 0.00 | 0.00 |
| | | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0.0 | 54 | 5.56 | 0.00 |
| | | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0.0 | 96 | 5.21 | 0.00 |
| | 5 | Average | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0.0 | 75 | 5.38 | 0.00 |
| | | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 72 | 1.39 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 75 | 0.00 | 0.00 |
| | 6 | Average | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.0 | 73.5 | 0.69 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 66 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 38 | 0.00 | 0.00 |
| | 7 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 52 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 86 | 0.00 | 0.00 |
| | | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0.0 | 70 | 5.71 | 0.00 |
| | 8 | Average | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.0 | 78 | 2.86 | 0.00 |
| | | 1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0.0 | 70 | 11.43 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 161 | 0.00 | 0.00 |
| | 9 | Average | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0.0 | 115.5 | 5.71 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 114 | 0.00 | 0.00 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.0 | 125 | 0.80 | 0.00 |
| | 10 | Average | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.0 | 119.5 | 0.40 | 0.00 |
| | Average | | 2.05 | 0.05 | 0.00 | 0.05 | 0.05 | 0.00 | 0.20 | 0.25 | 2.15 | 0.5 | 100.95 | 2.80 | 5.60 |
| 0.010 | SD | | 2.76 | 0.16 | 0.00 | 0.16 | 0.16 | 0.00 | 0.48 | 0.79 | 2.86 | 1.4 | 64.85 | 3.38 | 12.94 |

## *Appendix Table 8: 0.000 g/cm$^3$ Neem with $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 10 | 0 | 0 | 0 | 16 | 21 | 1 | 4 | 10 | 42 | 94 | 55.32 | 80.77 |
| | | 2 | 0 | 1 | 0 | 0 | 75 | 0 | 0 | 0 | 1 | 75 | 178 | 42.70 | 98.68 |
| | 1 | Average | 5 | 0.5 | 0 | 0 | 45.5 | 10.5 | 0.5 | 2 | 5.5 | 58.5 | 136 | 49.01 | 89.73 |
| | | 1 | 0 | 0 | 0 | 0 | 50 | 1 | 0 | 0 | 0 | 51 | 59 | 86.44 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 47 | 0 | 0 | 0 | 0 | 47 | 67 | 70.15 | 100.00 |
| | 2 | Average | 0 | 0 | 0 | 0 | 48.5 | 0.5 | 0 | 0 | 0 | 49 | 63 | 78.29 | 100.00 |
| | | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 154 | 1.95 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 12 | 79 | 15.19 | 100.00 |
| | 3 | Average | 0 | 0 | 0 | 0 | 7.5 | 0 | 0 | 0 | 0 | 7.5 | 116.5 | 8.57 | 100.00 |
| | | 1 | 0 | 0 | 0 | 0 | 87 | 0 | 0 | 0 | 0 | 87 | 140 | 62.14 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 23 | 64 | 35.94 | 100.00 |
| | 4 | Average | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 55 | 102 | 49.04 | 100.00 |
| | | 1 | 0 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 5 | 119 | 4.20 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 45 | | 0 | 0 | 0 | 45 | 56 | 80.36 | 100.00 |
| | 5 | Average | 0 | 0 | 0 | 0 | 23 | 4 | 0 | 0 | 0 | 25 | 87.5 | 42.28 | 100.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 102 | 0.98 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 4 | 125 | 3.20 | 100.00 |
| | 6 | Average | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 2 | 0 | 2.5 | 113.5 | 2.09 | 100.00 |
| | | 1 | 3 | 0 | 0 | 0 | 20 | 0 | 0 | 1 | 3 | 21 | 86 | 27.91 | 87.50 |
| | | 2 | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 4 | 98 | 8.16 | 50.00 |
| | 7 | Average | 3.5 | 0 | 0 | 0 | 12 | 0 | 0 | 0.5 | 3.5 | 12.5 | 92 | 18.04 | 68.75 |
| | | 1 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 107 | 3.74 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 139 | 2.88 | 100.00 |
| | 8 | Average | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 123 | 3.31 | 100.00 |
| | | 1 | 0 | 0 | 1 | 0 | 6 | 1 | 1 | 0 | 1 | 8 | 246 | 3.66 | 88.89 |
| | | 2 | 0 | 0 | 1 | 0 | 5 | 5 | 0 | 0 | 1 | 10 | 264 | 4.17 | 90.91 |
| | 9 | Average | 0 | 0 | 1 | 0 | 5.5 | 3 | 0.5 | 0 | 1 | 9 | 255 | 3.91 | 89.90 |
| | | 1 | 2 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 2 | 5 | 78 | 8.97 | 71.43 |
| | | 2 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 14 | 40 | 35.00 | 100.00 |
| | 10 | Average | 1 | 0 | 0 | 0 | 9 | 0.5 | 0 | 0 | 1 | 9.5 | 59 | 21.99 | 85.71 |
| | Average | | 0.95 | 0.05 | 0.10 | 0.00 | 21.00 | 1.90 | 0.10 | 0.45 | 1.10 | 23.25 | 114.75 | 27.65 | 93.41 |
| 0.0000 | SD | | 1.80 | 0.16 | 0.32 | 0.00 | 20.80 | 3.33 | 0.21 | 0.83 | 1.90 | 22.30 | 55.18 | 25.80 | 10.29 |

## *Appendix Table 9: 0.002 g/cm$^3$ Neem with $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 39.00 | 0.00 | 0.00 | 0.00 | 0 | 39 | 144 | 27.08 | 100.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 29.00 | 10.00 | 3.00 | 0.00 | 0 | 42 | 431 | 9.74 | 100.00 |
| | 1 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 34.00 | 5.00 | 1.50 | 0.00 | 0 | 40.5 | 287.5 | 18.41 | 100.00 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 27.00 | 0.00 | 0.00 | 1.00 | 0 | 28 | 91 | 30.77 | 100.00 |
| | | 2 | 1.00 | 0.00 | 0.00 | 0.00 | 29.00 | 1.00 | 0.00 | 0.00 | 1 | 30 | 135 | 22.96 | 96.77 |
| | 2 | Average | 0.50 | 0.00 | 0.00 | 0.00 | 28.00 | 0.50 | 0.00 | 0.50 | 0.5 | 29 | 113 | 26.87 | 98.39 |
| | | 1 | 0.00 | 1.00 | 0.00 | 0.00 | 8.00 | 0.00 | 1.00 | 1.00 | 1 | 10 | 265 | 4.15 | 90.91 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 | 1.00 | 0.00 | 0.00 | 0 | 3 | 147 | 2.04 | 100.00 |
| | 3 | Average | 0.00 | 0.50 | 0.00 | 0.00 | 5.00 | 0.50 | 0.50 | 0.50 | 0.5 | 6.5 | 206 | 3.10 | 95.45 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 24.00 | 0.00 | 1.00 | 0.00 | 0 | 25 | 158 | 15.82 | 100.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 10.00 | 0.00 | 0.00 | 0.00 | 0 | 10 | 128 | 7.81 | 100.00 |
| | 4 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 17.00 | 0.00 | 0.50 | 0.00 | 0 | 17.5 | 143 | 11.82 | 100.00 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 72 | 0.00 | 0.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 7.00 | 5.00 | 0.00 | 0.00 | 0 | 12 | 240 | 5.00 | 100.00 |
| | 5 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 3.50 | 2.50 | 0.00 | 0.00 | 0 | 6 | 156 | 2.50 | 50.00 |
| | | 1 | 23.00 | 0.00 | 0.00 | 0.00 | 24.00 | 0.00 | 0.00 | 0.00 | 23 | 24 | 213 | 22.07 | 51.06 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0 | 3 | 84 | 3.57 | 100.00 |
| | 6 | Average | 11.50 | 0.00 | 0.00 | 0.00 | 13.50 | 0.00 | 0.00 | 0.00 | 11.5 | 13.5 | 148.5 | 12.82 | 75.53 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0 | 1 | 83 | 1.20 | 100.00 |
| | | 2 | 5.00 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 5 | 3 | 147 | 5.44 | 37.50 |
| | 7 | Average | 2.50 | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 0.00 | 0.00 | 2.5 | 2 | 115 | 3.32 | 68.75 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0 | 1 | 105 | 0.95 | 100.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 98 | 0.00 | 0.00 |
| | 8 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.50 | 0.00 | 0 | 0.5 | 101.5 | 0.48 | 50.00 |
| | | 1 | 35.00 | 0.00 | 0.00 | 0.00 | 34.00 | 2.00 | 2.00 | 0.00 | 35 | 38 | 283 | 25.80 | 52.05 |
| | | 2 | 2.00 | 0.00 | 0.00 | 0.00 | 3.00 | 3.00 | 3.00 | 0.00 | 2 | 9 | 191 | 5.76 | 81.82 |
| | 9 | Average | 18.50 | 0.00 | 0.00 | 0.00 | 18.50 | 2.50 | 2.50 | 0.00 | 18.5 | 23.5 | 237 | 15.78 | 66.94 |
| | | 1 | 2.00 | 0.00 | 0.00 | 0.00 | 4.00 | 6.00 | 0.00 | 0.00 | 2 | 10 | 249 | 4.82 | 83.33 |
| | | 2 | 1.00 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 1 | 6 | 362 | 1.93 | 85.71 |
| | 10 | Average | 1.50 | 0.00 | 0.00 | 0.00 | 5.00 | 3.00 | 0.00 | 0.00 | 1.5 | 8 | 305.5 | 3.38 | 84.52 |
| | Average | | 3.45 | 0.05 | 0.00 | 0.00 | 12.65 | 1.40 | 0.55 | 0.10 | 3.50 | 14.70 | 181.30 | 9.85 | 78.96 |
| 0.002 | SD | | 6.36 | 0.16 | 0.00 | 0.00 | 11.64 | 1.74 | 0.83 | 0.21 | 6.33 | 12.94 | 73.81 | 8.71 | 19.73 |

## *Appendix Table 10: 0.004 g/cm$^3$ Neem with $H_2O_2$*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 5.00 | 1 | 7 | 42 | 19.05 | 87.50 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 68 | 0.00 | 0.00 |
| | 1 | Average | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 0.50 | 0.50 | 2.50 | 0.5 | 3.5 | 55 | 9.52 | 43.75 |
| | | 1 | 1.00 | 0.00 | 0.00 | 0.00 | 10.00 | 2.00 | 0.00 | 1.00 | 1 | 13 | 89 | 15.73 | 92.86 |
| | | 2 | 1.00 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 1.00 | 1 | 13 | 90 | 15.56 | 92.86 |
| | 2 | Average | 1.00 | 0.00 | 0.00 | 0.00 | 11.00 | 1.00 | 0.00 | 1.00 | 1 | 13 | 89.5 | 15.64 | 92.86 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 5.00 | 0.00 | 0.00 | 0.00 | 0 | 5 | 83 | 6.02 | 100.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 9.00 | 0.00 | 0.00 | 0.00 | 0 | 9 | 93 | 9.68 | 100.00 |
| | 3 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 7.00 | 0.00 | 0.00 | 0.00 | 0 | 7 | 88 | 7.85 | 100.00 |
| | | 1 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2 | 0 | 144 | 1.39 | 0.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.00 | 0.00 | 1.00 | 0 | 5 | 194 | 2.58 | 100.00 |
| | 4 | Average | 0.50 | 0.50 | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 0.50 | 1 | 2.5 | 169 | 1.98 | 50.00 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 257 | 0.00 | 0.00 |
| | | 2 | 1.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 1 | 1 | 256 | 0.78 | 50.00 |
| | 5 | Average | 0.50 | 0.00 | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 0.5 | 0.5 | 256.5 | 0.39 | 25.00 |
| | | 1 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 2.00 | 3.00 | 0.00 | 1 | 5 | 167 | 3.59 | 83.33 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 0 | 2 | 88 | 2.27 | 100.00 |
| | 6 | Average | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 1.00 | 2.50 | 0.00 | 0.5 | 3.5 | 127.5 | 2.93 | 91.67 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 137 | 0.00 | 0.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 160 | 0.00 | 0.00 |
| | 7 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 148.5 | 0.00 | 0.00 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 52 | 0.00 | 0.00 |
| | | 2 | 2.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 2 | 1 | 189 | 1.59 | 33.33 |
| | 8 | Average | 1.00 | 0.00 | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 1 | 0.5 | 120.5 | 0.79 | 16.67 |
| | | 1 | 0.00 | 1.00 | 0.00 | 0.00 | 1.00 | 1.00 | 3.00 | 0.00 | 1 | 5 | 180 | 3.33 | 83.33 |
| | | 2 | 1.00 | 0.00 | 0.00 | 0.00 | 11.00 | 0.00 | 0.00 | 0.00 | 1 | 11 | 165 | 7.27 | 91.67 |
| | 9 | Average | 0.50 | 0.50 | 0.00 | 0.00 | 6.00 | 0.50 | 1.50 | 0.00 | 1 | 8 | 172.5 | 5.30 | 87.50 |
| | | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 15.00 | 0.00 | 0.00 | 0.00 | 0 | 15 | 81 | 18.52 | 100.00 |
| | | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 19.00 | 0.00 | 0.00 | 0.00 | 0 | 19 | 148 | 12.84 | 100.00 |
| | 10 | Average | 0.00 | 0.00 | 0.00 | 0.00 | 17.00 | 0.00 | 0.00 | 0.00 | 0 | 17 | 114.5 | 15.68 | 100.00 |
| | Average | | 0.35 | 0.20 | 0.00 | 0.00 | 4.20 | 0.50 | 0.45 | 0.40 | 0.55 | 5.55 | 134.15 | 6.01 | 60.74 |
| 0.004 | SD | | 0.41 | 0.26 | 0.00 | 0.00 | 5.95 | 0.67 | 0.86 | 0.81 | 0.44 | 5.72 | 56.63 | 5.99 | 38.15 |

## Appendix Table 11: 0.006 g/cm$^3$ Neem with $H_2O_2$

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 6 | 0 | 0 | 0 | 11 | 2 | 0 | 0 | 6 | 13 | 170 | 11.18 | 68.42 |
| | | 2 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 94 | 5.32 | 100.00 |
| | 1 | Average | 3 | 0 | 0 | 0 | 8 | 1 | 0 | 0 | 3 | 9 | 132 | 8.25 | 84.21 |
| | | 1 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 | 6 | 115 | 9.57 | 54.55 |
| | | 2 | 13 | 0 | 0 | 0 | 12 | 1 | 0 | 2 | 13 | 15 | 117 | 23.93 | 53.57 |
| | 2 | Average | 9 | 0 | 0 | 0 | 8.5 | 0.5 | 0 | 1.5 | 9 | 10.5 | 116 | 16.75 | 54.06 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 159 | 0.63 | 100.00 |
| | | 2 | 1 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 1 | 17 | 94 | 19.15 | 94.44 |
| | 3 | Average | 0.5 | 0 | 0 | 0 | 8.5 | 0 | 0.5 | 0 | 0.5 | 9 | 126.5 | 9.89 | 97.22 |
| | | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 114 | 0.88 | 0.00 |
| | | 2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 83 | 0.00 | 0.00 |
| | 4 | Average | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 98.5 | 0.44 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 5 | 141 | 3.55 | 100.00 |
| | | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 4 | 1 | 82 | 6.10 | 20.00 |
| | 5 | Average | 0 | 1 | 1 | 0 | 0 | 2 | 1 | 0 | 2 | 3 | 111.5 | 4.82 | 60.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 52 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 87 | 2.30 | 100.00 |
| | 6 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 69.5 | 1.15 | 50.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 164 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 154 | 0.00 | 0.00 |
| | 7 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 159 | 0.00 | 0.00 |
| | | 1 | 5 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 9 | 0 | 103 | 8.74 | 0.00 |
| | | 2 | 9 | 2 | 1 | | 3 | 1 | | 1 | 12 | 5 | 280 | 6.07 | 29.41 |
| | 8 | Average | 7 | 1.5 | 1.5 | 1 | 1.5 | 0.5 | 0 | 0.5 | 10.5 | 2.5 | 191.5 | 7.40 | 14.71 |
| | | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 326 | 0.92 | 66.67 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 337 | 0.00 | 0.00 |
| | 9 | Average | 0.5 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0 | 0.5 | 1 | 331.5 | 0.46 | 33.33 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 128 | 0.00 | 0.00 |
| | | 2 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 1 | 70 | 7.14 | 20.00 |
| | 10 | Average | 2 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 2 | 0.5 | 99 | 3.57 | 10.00 |
| | Average | | 2.30 | 0.25 | 0.25 | 0.10 | 2.70 | 0.45 | 0.30 | 0.20 | 2.80 | 3.65 | 143.50 | 5.27 | 40.35 |
| 0.006 | SD | | 3.19 | 0.54 | 0.54 | 0.32 | 3.92 | 0.64 | 0.42 | 0.48 | 3.81 | 4.17 | 74.22 | 5.38 | 34.50 |

## Appendix Table 12: 0.008 g/cm$^3$ Neem with $H_2O_2$

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 42 | 2.38 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0.00 | 0.00 |
| | 1 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.5 | 48.5 | 1.19 | 50.00 |
| | | 1 | 16 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 16 | 3 | 338 | 5.62 | 15.79 |
| | | 2 | 11 | 0 | 0 | 0 | 8 | 1 | 0 | 1 | 11 | 10 | 122 | 17.21 | 47.62 |
| | 2 | Average | 13.5 | 0 | 0 | 0 | 4.5 | 1 | 0 | 1 | 13.5 | 6.5 | 230 | 11.42 | 31.70 |
| | | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | 122 | 3.28 | 25.00 |
| | | 2 | 13 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 13 | 5 | 130 | 13.85 | 27.78 |
| | 3 | Average | 8 | 0 | 0 | 0 | 2.5 | 0 | 0.5 | 0 | 8 | 3 | 126 | 8.56 | 26.39 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 72 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 109 | 0.00 | 0.00 |
| | 4 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 90.5 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 92 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 83 | 0.00 | 0.00 |
| | 5 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 87.5 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 113 | 0.00 | 0.00 |
| | 6 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 98 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 189 | 0.00 | 0.00 |
| | 7 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 143.5 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 146 | 0.00 | 0.00 |
| | | 2 | 8 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 8 | 3 | 131 | 8.40 | 27.27 |
| | 8 | Average | 4 | 0 | 0 | 0 | 1.5 | 0 | 0 | 0 | 4 | 1.5 | 138.5 | 4.20 | 13.64 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 130 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 236 | 0.00 | 0.00 |
| | 9 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 183 | 0.00 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 263 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 163 | 0.00 | 0.00 |
| | 10 | Average | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 213 | 0.00 | 0.00 |
| | Average | | 2.55 | 0.00 | 0.00 | 0.00 | 0.85 | 0.10 | 0.05 | 0.15 | 2.55 | 1.15 | 135.65 | 2.54 | 12.17 |
| 0.008 | SD | | 4.68 | 0.00 | 0.00 | 0.00 | 1.55 | 0.32 | 0.16 | 0.34 | 4.68 | 2.12 | 58.51 | 4.19 | 17.97 |

## Appendix Table 13: 0.010 g/cm$^3$ Neem with $H_2O_2$

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 8 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 8 | 1 | 45 | 20.00 | 11.11 |
| | | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 80 | 5.00 | 0.00 |
| | 1 | Average | 6 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 6 | 0.5 | 62.5 | 12.50 | 5.56 |
| | | 1 | 10 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 10 | 4 | 299 | 4.68 | 28.57 |
| | | 2 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 108 | 7.41 | 0.00 |
| | 2 | Average | 9 | 0 | 0 | 0 | 1 | 0.5 | 0 | 0.5 | 9 | 2 | 203.5 | 6.04 | 14.29 |
| | | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 100 | 2.00 | 100.00 |
| | | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 374 | 0.80 | 66.67 |
| | 3 | Average | 0.5 | 0 | 0 | 0 | 1.5 | 0 | 0.5 | 0 | 0.5 | 2 | 237 | 1.40 | 83.33 |
| | | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | 157 | 2.55 | 25.00 |
| | | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 227 | 0.88 | 50.00 |
| | 4 | Average | 0.5 | 1.5 | 0 | 0 | 0.5 | 0 | 0.5 | 0 | 2 | 1 | 192 | 1.71 | 37.50 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 117 | 0.00 | 0.00 |
| | | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 70 | 8.57 | 0.00 |
| | 5 | Average | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 93.5 | 4.29 | 0.00 |
| | | 1 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 230 | 3.91 | 0.00 |
| | | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 130 | 1.54 | 0.00 |
| | 6 | Average | 5.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.5 | 0 | 180 | 2.73 | 0.00 |
| | | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 276 | 0.36 | 0.00 |
| | | 2 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 37 | 0 | 128 | 28.91 | 0.00 |
| | 7 | Average | 18.5 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 202 | 14.63 | 0.00 |
| | | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 111 | 0.90 | 0.00 |
| | | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 245 | 2.04 | 0.00 |
| | 8 | Average | 2.5 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 178 | 1.47 | 0.00 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 137 | 0.00 | 0.00 |
| | | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 173 | 1.16 | 0.00 |
| | 9 | Average | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 155 | 0.58 | 0.00 |
| | | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 116 | 1.72 | 0.00 |
| | | 2 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 174 | 6.32 | 0.00 |
| | 10 | Average | 6.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.5 | 0 | 145 | 4.02 | 0.00 |
| | Average | | 5.30 | 0.25 | 0.00 | 0.00 | 0.35 | 0.05 | 0.10 | 0.05 | 5.55 | 0.55 | 164.85 | 4.94 | 14.07 |
| 0.010 | SD | | 5.45 | 0.49 | 0.00 | 0.00 | 0.53 | 0.16 | 0.21 | 0.16 | 5.43 | 0.83 | 53.00 | 2.11 | 27.09 |

## 8.4 ANOVA TEST AND TUKEY TEST (FULL)

### 8.4.1 *Neem without $H_2O_2$: A-MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up without $H_2O_2$ and A-MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up without $H_2O_2$ and A-MI.

| Anova: Single Factor Neem w/o H2O2 Abnormal MI | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| 0 | 10 | 245.16 | 24.516 | 329.6946 | | |
| 0.002 | 10 | 145.35 | 14.535 | 236.7192 | | |
| 0.004 | 10 | 173.57 | 17.357 | 664.2271 | | |
| 0.006 | 10 | 145.83 | 14.583 | 691.5556 | | |
| 0.008 | 10 | 5.77 | 0.577 | 3.32929 | | |
| 0.01 | 10 | 55.96 | 5.596 | 167.6045 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 3654.975 | 5 | 730.995 | 2.095412 | 0.080053 | 2.38607 |
| Within Groups | 18838.17 | 54 | 348.855 | | | |
| | | | | | | |
| Total | 22493.15 | 59 | | | | |

Null Hypothesis accepted. Hence no Tukey test was done.

### 8.4.2 *Neem with $H_2O_2$: MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and MI.

| Anova: Single Factor Neem w H2O2 MI | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| 0 | 10 | 276.53 | 27.653 | 665.5554 | | |
| 0.002 | 10 | 98.48 | 9.848 | 75.85604 | | |
| 0.004 | 10 | 60.08 | 6.008 | 35.90397 | | |
| 0.006 | 10 | 52.73 | 5.273 | 28.98409 | | |
| 0.008 | 10 | 25.37 | 2.537 | 17.59805 | | |
| 0.01 | 10 | 49.37 | 4.937 | 23.60307 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 4289.262 | 5 | 857.8524 | 6.073286 | 0.000157 | 2.38607 |
| Within Groups | 7627.506 | 54 | 141.2501 | | | |
| | | | | | | |
| Total | 11916.77 | 59 | | | | |

Null hypothesis was rejected. Tukey test was done. Q critical values are the same as mentioned above.

| Comparisons | MI for set up with neem with $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | |
| 0.000 vs 0.002 | 4.7375 | 0.0175975 | * p<0.05 |
| 0.000 vs 0.004 | 5.7592 | 0.0020292 | ** p<0.01 |
| 0.000 vs 0.006 | 5.9548 | 0.0013008 | ** p<0.01 |
| 0.000 vs 0.008 | 6.6828 | 0.0010053 | ** p<0.01 |
| 0.000 vs 0.010 | 6.0442 | 0.0010594 | ** p<0.01 |
| 0.002 vs 0.004 | 1.0217 | 0.8999947 | insignificant |
| 0.002 vs 0.006 | 1.2173 | 0.8999947 | insignificant |
| 0.002 vs 0.008 | 1.9453 | 0.7154867 | insignificant |
| 0.002 vs 0.010 | 1.3067 | 0.8999947 | insignificant |
| 0.004 vs 0.006 | 0.1956 | 0.8999947 | insignificant |
| 0.004 vs 0.008 | 0.9235 | 0.8999947 | insignificant |
| 0.004 vs 0.010 | 0.285 | 0.8999947 | insignificant |
| 0.006 vs 0.008 | 0.728 | 0.8999947 | insignificant |
| 0.006 vs 0.010 | 0.0894 | 0.8999947 | insignificant |
| 0.008 vs 0.010 | 0.6386 | 0.8999947 | insignificant |

### 8.4.3 *Neem with $H_2O_2$: A-MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and A-MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and A-MI.

| Anova: Single Factor Neem w H2O2 Abnormal MI | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| 0 | 10 | 934.09 | 93.409 | 105.9819 | | |
| 0.002 | 10 | 789.58 | 78.958 | 389.286 | | |
| 0.004 | 10 | 607.45 | 60.745 | 1455.558 | | |
| 0.006 | 10 | 403.53 | 40.353 | 1189.907 | | |
| 0.008 | 10 | 121.73 | 12.173 | 322.8392 | | |
| 0.01 | 10 | 140.68 | 14.068 | 734.0189 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 56534.03 | 5 | 11306.81 | 16.16185 | 1.02E-09 | 2.38607 |
| Within Groups | 37778.32 | 54 | 699.5985 | | | |
| | | | | | | |
| Total | 94312.34 | 59 | | | | |

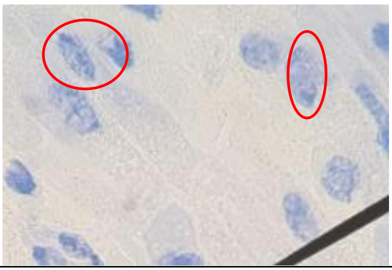
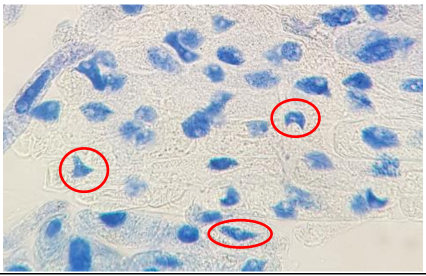
Null Hypothesis was rejected. Tukey test was done. Q critical values are the same as mentioned above.

| Comparisons | A-MI for set up with neem with $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | |
| 0.000 vs 0.002 | 1.7277 | 0.8020583 | insignificant |
| 0.000 vs 0.004 | 3.9052 | 0.0799202 | insignificant |
| 0.000 vs 0.006 | 6.3432 | 0.0010053 | ** p<0.01 |
| 0.000 vs 0.008 | 9.7123 | 0.0010053 | ** p<0.01 |
| 0.000 vs 0.010 | 9.4858 | 0.0010053 | ** p<0.01 |
| 0.002 vs 0.004 | 2.1775 | 0.6230857 | insignificant |
| 0.002 vs 0.006 | 4.6155 | 0.0223118 | * p<0.05 |
| 0.002 vs 0.008 | 7.9846 | 0.0010053 | ** p<0.01 |
| 0.002 vs 0.010 | 7.7581 | 0.0010053 | ** p<0.01 |
| 0.004 vs 0.006 | 2.438 | 0.5194205 | insignificant |
| 0.004 vs 0.008 | 5.8071 | 0.0018221 | ** p<0.01 |
| 0.004 vs 0.010 | 5.5806 | 0.0030187 | ** p<0.01 |
| 0.006 vs 0.008 | 3.3691 | 0.1806194 | insignificant |
| 0.006 vs 0.010 | 3.1426 | 0.2446772 | insignificant |
| 0.008 vs 0.010 | 0.2266 | 0.8999947 | insignificant |

# 9 PRELIMINARY RESULTS FOR OTHER ABNORMALITIES AFTER 24H

## 9.1 QUALITATIVE DATA

*Appendix Table 14: Types of abnormalities*

| Type of abnormality | Photos taken by me | Description |
|---|---|---|
| Apoptosis |  | Complete disintegration of the nucleus |
| Fragmentation of the nucleus |  | The blue intensity of the dye is very minimal and it is not concentrated around one area rather it is spread out unevenly. |
| Abnormally shaped nucleus |  | Not round and ideal nucleus. There are pointed edges and they looked slightly deformed. |

## 9.2 QUANTITATIVE DATA

*Appendix Table 15: Average type of abnormality for 10 replicates used in the main investigation*

| | | Types of abnormalities per 100 cells | | | |
|---|---|---|---|---|---|
| Group | Concentration | apoptosis | fragmentation of the nucleus | abnormally shaped nucleus | Total abnormalities |
| Neem without $H_2O_2$ | 0.000 | 1.21 | 0.11 | 0.00 | 1.32 |
| | 0.002 | 0.25 | 0.00 | 0.00 | 0.25 |
| | 0.004 | 0.12 | 0.32 | 0.00 | 0.44 |
| | 0.006 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 0.008 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 0.010 | 0.00 | 0.15 | 0.00 | 0.15 |
| Neem with $H_2O_2$ | 0.000 | 2.48 | 0.26 | 3.88 | 6.62 |
| | 0.002 | 0.03 | 1.13 | 0.30 | 1.46 |
| | 0.004 | 0.00 | 0.71 | 4.73 | 5.44 |
| | 0.006 | 0.21 | 0.10 | 0.66 | 0.98 |
| | 0.008 | 0.00 | 0.07 | 0.11 | 0.18 |
| | 0.010 | 3.12 | 0.09 | 0.03 | 3.25 |

## 9.3 DISCUSSION

The average total number of abnormal figures in the group without $H_2O_2$ show a decreasing trend. However, since the average number of abnormal figures per 100 cell is very small, any difference in the number of abnormal figures cannot be confidently attributed to the effects of neem. In the group with $H_2O_2$, there is a decreasing trend in the total number of abnormal figures with a few anomalies (in red), reflecting the main investigation.

After a series of cascades, the nucleus fragments, cytoskeleton gets disrupted, the membrane starts to bleb and the cell fragments[24,34], possibly triggering apoptosis.

However, this may occur at a much lower extent in the absence of oxidative stress. Hence the results show no trend in the absence of $H_2O_2$. This could indicate neem's specificity when it comes to causing programmed cell death and acting on cells that actually undergo oxidative stress.

Both part 1 and part 2 showed that within 24h results can be seen. Part 3 was done to see if the effects lasted 48h.

# 10 PRELIMINARY RESULTS FOR INVESTIGATING MI AFTER 48H

## 10.1 RAW DATA FOR PART 2

*Appendix Table 16: MI after 48h for set up with neem without hydrogen peroxide*

| Mitotic Index | Concentration | Replicate | Prophase | Metaphase | Anaphase | Telophase | A-Prophase | A-metaphase | A-Anaphase | A-telophase | Total number of cells in normal mitosis | Total number of cells in Abnormal Mitosis | Total Number of cells | MI-N | MI-A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 12 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 13 | 1 | 86 | 16.28 | 7.14 |
| | | 2 | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 4 | 2 | 70 | 8.57 | 33.33 |
| | | 3 | 34 | 1 | 0 | 0 | 3 | 2 | 0 | 4 | 35 | 9 | 230 | 19.13 | 20.45 |
| | | 4 | 14 | 0 | 1 | 6 | 12 | 1 | 0 | 0 | 21 | 13 | 237 | 14.35 | 38.24 |
| | | 5 | 15 | 0 | 0 | 0 | 1 | 0 | 4 | 0 | 15 | 5 | 89 | 22.47 | 25.00 |
| | | Average | 15.80 | 0.20 | 0.20 | 1.40 | 3.60 | 0.80 | 0.80 | 0.80 | 17.60 | 6.00 | 142.40 | 16.16 | 24.83 |
| | 0.000 | SD | 11.05 | 0.45 | 0.45 | 2.61 | 4.83 | 0.84 | 1.79 | 1.79 | 11.48 | 5.00 | 83.51 | 5.23 | 12.08 |
| | | 1 | 7 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 7 | 9 | 130 | 12.31 | 56.25 |
| | | 2 | 15 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 15 | 2 | 145 | 11.72 | 11.76 |
| | | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 59 | 1.69 | 0.00 |
| | | 4 | 18 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 18 | 1 | 116 | 16.38 | 5.26 |
| | | 5 | 12 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 13 | 2 | 97 | 15.46 | 13.33 |
| | | Average | 10.60 | 0.20 | 0.00 | 0.00 | 2.60 | 0.20 | 0.00 | 0.00 | 10.80 | 2.80 | 109.40 | 11.51 | 17.32 |
| | 0.002 | SD | 6.73 | 0.45 | 0.00 | 0.00 | 3.65 | 0.45 | 0.00 | 0.00 | 6.80 | 3.56 | 33.28 | 5.84 | 22.40 |
| | | 1 | 19 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 19 | 2 | 95 | 22.11 | 9.52 |
| | | 2 | 17 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 17 | 3 | 109 | 18.35 | 15.00 |
| | | 3 | 11 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 11 | 6 | 102 | 16.67 | 35.29 |
| | | 4 | 7 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 7 | 3 | 61 | 16.39 | 30.00 |
| | | 5 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 70 | 8.57 | 0.00 |
| | | Average | 12.00 | 0.00 | 0.00 | 0.00 | 2.80 | 0.00 | 0.00 | 0.00 | 12.00 | 2.80 | 87.40 | 16.42 | 17.96 |
| | 0.004 | SD | 5.83 | 0.00 | 0.00 | 0.00 | 2.17 | 0.00 | 0.00 | 0.00 | 5.83 | 2.17 | 20.84 | 4.94 | 14.56 |
| | | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 58 | 5.17 | 0.00 |
| | | 2 | 51 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 51 | 4 | 198 | 27.78 | 7.27 |
| | | 3 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 97 | 16.49 | 0.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 34 | 0.00 | 0.00 |
| | | 5 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 0 | 102 | 25.49 | 0.00 |
| | | Average | 19.20 | 0.00 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.40 | 19.20 | 0.80 | 97.80 | 14.99 | 1.45 |
| | 0.006 | SD | 20.61 | 0.00 | 0.00 | 0.00 | 0.89 | 0.00 | 0.00 | 0.89 | 20.61 | 1.79 | 62.68 | 12.22 | 3.25 |
| | | 1 | 33 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 33 | 3 | 103 | 34.95 | 8.33 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 57 | 0.00 | 0.00 |
| | | 3 | 47 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 49 | 0 | 246 | 19.92 | 0.00 |
| | | 4 | 30 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 30 | 3 | 95 | 34.74 | 9.09 |
| | | 5 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 71 | 21.13 | 0.00 |
| | | Average | 25.00 | 0.20 | 0.20 | 0.00 | 0.80 | 0.20 | 0.00 | 0.20 | 25.40 | 1.20 | 114.40 | 22.15 | 3.48 |
| | 0.008 | SD | 18.01 | 0.45 | 0.45 | 0.00 | 1.30 | 0.45 | 0.00 | 0.45 | 18.64 | 1.64 | 75.83 | 14.31 | 4.78 |
| | | 1 | 49 | 0 | 0 | 0 | 6 | 1 | 0 | 2 | 49 | 9 | 114 | 50.88 | 15.52 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 92 | 0.00 | 0.00 |
| | | 3 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 163 | 6.75 | 0.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 0.00 | 0.00 |
| | | 5 | 8 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 8 | 1 | 59 | 15.25 | 11.11 |
| | | Average | 13.60 | 0.00 | 0.00 | 0.00 | 1.40 | 0.20 | 0.00 | 0.40 | 13.60 | 2.00 | 99.60 | 14.58 | 5.33 |
| Neem | 0.010 | SD | 20.38 | 0.00 | 0.00 | 0.00 | 2.61 | 0.45 | 0.00 | 0.89 | 20.38 | 3.94 | 41.26 | 21.24 | 7.46 |

*Appendix Table 17: MI after 48h for set up with neem with hydrogen peroxide*

| Mitotic Index | Concentration | Replicate | Prophase | Metaphase | Anaphase | Telophase | A-Prophase | A-metaphase | A-Anaphase | A-telophase | Total number of cells in normal mitosis | Total number of cells in Abnormal Mitosis | Total Number of cells | MI-N | MI-A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 0 | 0 | 0 | 0 | 48 | 11 | 2 | 15 | 0 | 76 | 121 | 62.81 | 100.00 |
| | | 2 | 2 | 0 | 2 | 0 | 17 | 9 | 5 | 7 | 4 | 38 | 123 | 34.15 | 90.48 |
| | | 3 | 1 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 1 | 40 | 58 | 70.69 | 97.56 |
| | | 4 | 0 | 0 | 0 | 0 | 33 | 1 | 0 | 1 | 0 | 35 | 47 | 74.47 | 100.00 |
| | | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 41 | 12.20 | 100.00 |
| | | Average | 0.60 | 0.00 | 0.40 | 0.00 | 28.60 | 4.20 | 1.40 | 4.60 | 1.00 | 38.80 | 78.00 | 50.86 | 97.61 |
| | 0.000 | SD | 0.89 | 0.00 | 0.89 | 0.00 | 17.44 | 5.36 | 2.19 | 6.50 | 1.73 | 25.21 | 40.63 | 26.78 | 4.12 |
| | | 1 | 0 | 0 | 0 | 0 | 38 | 0 | 0 | 0 | 0 | 38 | 43 | 88.37 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 4 | 145 | 2.76 | 100.00 |
| | | 3 | 5 | 0 | 0 | 0 | 16 | 0 | 0 | 4 | 5 | 20 | 128 | 19.53 | 80.00 |
| | | 4 | 2 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 2 | 13 | 101 | 14.85 | 86.67 |
| | | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 5 | 52 | 9.62 | 100.00 |
| | | Average | 1.40 | 0.00 | 0.00 | 0.00 | 14.00 | 0.00 | 1.20 | 0.80 | 1.40 | 16.00 | 93.80 | 27.03 | 93.33 |
| | 0.002 | SD | 2.19 | 0.00 | 0.00 | 0.00 | 14.95 | 0.00 | 1.79 | 1.79 | 2.19 | 13.91 | 45.20 | 34.86 | 9.43 |
| | | 1 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 15 | 0 | 48 | 55 | 87.27 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 14 | 0 | 64 | 79 | 81.01 | 100.00 |
| | | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 94 | 3.19 | 66.67 |
| | | 4 | 1 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 10 | 9 | 79 | 24.05 | 47.37 |
| | | 5 | 1 | 0 | 0 | 0 | 2 | 0 | 4 | 0 | 1 | 6 | 61 | 11.48 | 85.71 |
| | | Average | 0.60 | 0.00 | 0.00 | 0.00 | 19.00 | 0.00 | 0.80 | 6.00 | 2.40 | 25.80 | 73.60 | 41.40 | 79.95 |
| | 0.004 | SD | 0.55 | 0.00 | 0.00 | 0.00 | 21.62 | 0.00 | 1.79 | 7.78 | 4.28 | 28.25 | 15.65 | 39.78 | 22.78 |
| | | 1 | 2 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 2 | 4 | 142 | 4.23 | 66.67 |
| | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 114 | 1.75 | 50.00 |
| | | 3 | 17 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 17 | 3 | 224 | 8.93 | 15.00 |
| | | 4 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 5 | 3 | 97 | 8.25 | 37.50 |
| | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 97 | 0.00 | 0.00 |
| | | Average | 4.40 | 0.00 | 0.00 | 0.00 | 0.80 | 0.60 | 0.00 | 0.80 | 5.00 | 2.20 | 134.80 | 4.63 | 33.83 |
| | 0.006 | SD | 7.09 | 0.00 | 0.00 | 0.00 | 0.84 | 1.34 | 0.00 | 0.84 | 6.96 | 1.64 | 53.15 | 3.92 | 26.70 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0.00 | 0.00 |
| | | 2 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 186 | 8.60 | 0.00 |
| | | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 155 | 0.65 | 0.00 |
| | | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 203 | 0.49 | 0.00 |
| | | 5 | 7 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 7 | 4 | 84 | 13.10 | 36.36 |
| | | Average | 5.00 | 0.00 | 0.00 | 0.00 | 0.80 | 0.00 | 0.00 | 0.00 | 5.00 | 0.80 | 145.60 | 4.57 | 7.27 |
| | 0.008 | SD | 6.75 | 0.00 | 0.00 | 0.00 | 1.79 | 0.00 | 0.00 | 0.00 | 6.75 | 1.79 | 52.18 | 5.96 | 16.26 |
| | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 125 | 0.00 | 0.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 52 | 0.00 | 0.00 |
| | | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 140 | 0.71 | 0.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 0.00 | 0.00 |
| | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0.00 | 0.00 |
| | | Average | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 82.40 | 0.14 | 0.00 |
| Neem in H2O2 | 0.010 | SD | 0.00 | 0.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.45 | 0.00 | 46.08 | 0.32 | 0.00 |

## 10.2 Processed Data

The data was obtained from 5 of the replicates in part 1 that were left longer for 48h. Detailed data is in the appendix.

*Appendix Table 18: MI of Neem without $H_2O_2$*

| Concentration / g/cm$^3$ | | 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |
|---|---|---|---|---|---|---|---|
| Replicates | 1 | 16.28 | 12.31 | 22.11 | 5.17 | 34.95 | 50.88 |
| | 2 | 8.57 | 11.72 | 18.35 | 27.78 | 0.00 | 0.00 |
| | 3 | 19.13 | 1.69 | 16.67 | 16.49 | 19.92 | 6.75 |
| | 4 | 14.35 | 16.38 | 16.39 | 0.00 | 34.74 | 0.00 |
| | 5 | 22.47 | 15.46 | 8.57 | 25.49 | 21.13 | 15.25 |

*Appendix Table 19: A-MI of Neem without $H_2O_2$*

| Concentration / g/cm$^3$ | | 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |
|---|---|---|---|---|---|---|---|
| Replicates | 1 | 7.14 | 56.25 | 9.52 | 0.00 | 8.33 | 15.52 |
| | 2 | 33.33 | 11.76 | 15.00 | 7.27 | 0.00 | 0.00 |
| | 3 | 20.45 | 0.00 | 35.29 | 0.00 | 0.00 | 0.00 |
| | 4 | 38.24 | 5.26 | 30.00 | 0.00 | 9.09 | 0.00 |
| | 5 | 25.00 | 13.33 | 0.00 | 0.00 | 0.00 | 11.11 |

*Appendix Table 20: MI: Neem with $H_2O_2$*

| Concentration / g/cm$^3$ | | 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |
|---|---|---|---|---|---|---|---|
| Replicates | 1 | 62.81 | 88.37 | 87.27 | 4.23 | 0.00 | 0.00 |
| | 2 | 34.15 | 2.76 | 81.01 | 1.75 | 8.60 | 0.00 |
| | 3 | 70.69 | 19.53 | 3.19 | 8.93 | 0.65 | 0.71 |
| | 4 | 74.47 | 14.85 | 24.05 | 8.25 | 0.49 | 0.00 |
| | 5 | 12.20 | 9.62 | 11.48 | 0.00 | 13.10 | 0.00 |

*Appendix Table 21: A-MI of Neem with $H_2O_2$*

| Concentration / g/cm$^3$ | | 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |
|---|---|---|---|---|---|---|---|
| Replicates | 1 | 100.00 | 100.00 | 100.00 | 66.67 | 0.00 | 0.00 |
| | 2 | 90.48 | 100.00 | 100.00 | 50.00 | 0.00 | 0.00 |
| | 3 | 97.56 | 80.00 | 66.67 | 15.00 | 0.00 | 0.00 |
| | 4 | 100.00 | 86.67 | 47.37 | 37.50 | 0.00 | 0.00 |
| | 5 | 100.00 | 100.00 | 85.71 | 0.00 | 36.36 | 0.00 |

## 10.3 DATA ANALYSIS

### *10.3.1 Neem without $H_2O_2$: MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up without $H_2O_2$ and the MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up without $H_2O_2$ and the MI.

| Anova: Single Factor Neem w/o H2O2 MI after 48h | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| 0 | 5 | 80.79883 | 16.15977 | 27.38873 | | |
| 0.002 | 5 | 57.56997 | 11.51399 | 34.09041 | | |
| 0.004 | 5 | 82.08542 | 16.41708 | 24.42585 | | |
| 0.006 | 5 | 74.93523 | 14.98705 | 149.2827 | | |
| 0.008 | 5 | 110.7338 | 22.14675 | 204.7385 | | |
| 0.01 | 5 | 72.8799 | 14.57598 | 451.1066 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 305.7678 | 5 | 61.15357 | 0.411793 | 0.835824 | 2.620654 |
| Within Groups | 3564.131 | 24 | 148.5055 | | | |
| | | | | | | |
| Total | 3869.899 | 29 | | | | |

Since the p value is more than 0.05, the null hypothesis was accepted.

### 10.3.2 *Neem without $H_2O_2$: A-MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up without $H_2O_2$ and the A-MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up without $H_2O_2$ and the A-MI.

| Anova: Single Factor Neem w/o H2O2 Abnormal MI after 48h | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| 0 | 5 | 124.166 | 24.83321 | 146.0043 | | |
| 0.002 | 5 | 86.6112 | 17.32224 | 501.9124 | | |
| 0.004 | 5 | 89.81793 | 17.96359 | 211.9814 | | |
| 0.006 | 5 | 7.272727 | 1.454545 | 10.57851 | | |
| 0.008 | 5 | 17.42424 | 3.484848 | 22.84206 | | |
| 0.01 | 5 | 26.62835 | 5.32567 | 55.60693 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 2282.038 | 5 | 456.4077 | 2.885839 | 0.035330266 | 2.620654 |
| Within Groups | 3795.702 | 24 | 158.1543 | | | |
| | | | | | | |
| Total | 6077.741 | 29 | | | | |

Null Hypothesis accepted. No Tukey test was done.

### 10.3.3 *Neem with $H_2O_2$: MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and the MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and the MI.

| Anova: Single Factor Neem w H2O2 MI after 48h | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| 0 | 5 | 254.3091 | 50.86182 | 716.9191 | | |
| 0.002 | 5 | 135.1288 | 27.02577 | 1214.942 | | |
| 0.004 | 5 | 207.0029 | 41.40058 | 1582.46 | | |
| 0.006 | 5 | 23.15573 | 4.631146 | 15.35831 | | |
| 0.008 | 5 | 22.83516 | 4.567032 | 35.46306 | | |
| 0.01 | 5 | 0.714286 | 0.142857 | 0.102041 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 11580.36 | 5 | 2316.072 | 3.89775 | 0.009968 | 2.620654 |
| Within Groups | 14260.98 | 24 | 594.2075 | | | |
| | | | | | | |
| Total | 25841.34 | 29 | | | | |

Null hypothesis was rejected. Tukey test was done and the Q critical values are as follows:

$Q_{\alpha=0.01,k=6,\nu=24}$critical = 5.3738 $Q_{\alpha=0.05,k=6,\nu=24}$critical = 4.3729

| Comparisons | MI for Neem with $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | |
| 0.000 vs 0.002 | 2.1865 | 0.6232802 | insignificant |
| 0.000 vs 0.004 | 0.8679 | 0.8999947 | insignificant |
| 0.000 vs 0.006 | 4.2408 | 0.061082 | insignificant |
| 0.000 vs 0.008 | 4.2467 | 0.0605443 | insignificant |
| 0.000 vs 0.010 | 4.6525 | 0.0324203 | * p<0.05 |
| 0.002 vs 0.004 | 1.3186 | 0.8999947 | insignificant |
| 0.002 vs 0.006 | 2.0543 | 0.6741045 | insignificant |
| 0.002 vs 0.008 | 2.0602 | 0.671843 | insignificant |
| 0.002 vs 0.010 | 2.466 | 0.515844 | insignificant |
| 0.004 vs 0.006 | 3.3729 | 0.2010785 | insignificant |
| 0.004 vs 0.008 | 3.3788 | 0.199612 | insignificant |
| 0.004 vs 0.010 | 3.7846 | 0.117627 | insignificant |
| 0.006 vs 0.008 | 0.0059 | 0.8999947 | insignificant |
| 0.006 vs 0.010 | 0.4117 | 0.8999947 | insignificant |
| 0.008 vs 0.010 | 0.4058 | 0.8999947 | insignificant |

### *10.3.4 Neem with $H_2O_2$: A-MI*

**Null hypothesis:** There is no relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and the A-MI.

**Alternative hypothesis:** There is a relationship between increasing concentrations of neem in the set-up with $H_2O_2$ and the A-MI.

| Anova: Single Factor | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| 0 | 5 | 488.0372 | 97.60743 | 17.00748 | | |
| 0.002 | 5 | 466.6667 | 93.33333 | 88.88889 | | |
| 0.004 | 5 | 399.7494 | 79.94987 | 518.8096 | | |
| 0.006 | 5 | 169.1667 | 33.83333 | 713.0556 | | |
| 0.008 | 5 | 36.36364 | 7.272727 | 264.4628 | | |
| 0.01 | 5 | 0 | 0 | 0 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 48021.17 | 5 | 9604.235 | 35.96588 | 2.16714E-10 | 2.620654 |
| Within Groups | 6408.897 | 24 | 267.0374 | | | |
| | | | | | | |
| Total | 54430.07 | 29 | | | | |

Null Hypothesis was rejected Tukey test was done. Q critical values are the same as mentioned above.

| Comparisons | A-MI for set-up with $H_2O_2$ | | |
|---|---|---|---|
| | Tukey HSD Q statistics | Tukey HSD p value | |
| 0.000 vs 0.002 | 0.5848 | 0.8999947 | Insignificant |
| 0.000 vs 0.004 | 2.4162 | 0.5349923 | Insignificant |
| 0.000 vs 0.006 | 8.7266 | 0.0010053 | ** $p<0.01$ |
| 0.000 vs 0.008 | 12.361 | 0.0010053 | ** $p<0.01$ |
| 0.000 vs 0.010 | 13.3562 | 0.0010053 | ** $p<0.01$ |
| 0.002 vs 0.004 | 1.8313 | 0.7598008 | insignificant |
| 0.002 vs 0.006 | 8.1417 | 0.0010053 | ** $p<0.01$ |
| 0.002 vs 0.008 | 11.7762 | 0.0010053 | ** $p<0.01$ |
| 0.002 vs 0.010 | 12.7713 | 0.0010053 | ** $p<0.01$ |
| 0.004 vs 0.006 | 6.3104 | 0.0020158 | ** $p<0.01$ |
| 0.004 vs 0.008 | 9.9448 | 0.0010053 | ** $p<0.01$ |
| 0.004 vs 0.010 | 10.94 | 0.0010053 | ** $p<0.01$ |
| 0.006 vs 0.008 | 3.6344 | 0.1437284 | insignificant |
| 0.006 vs 0.010 | 4.6296 | 0.0336115 | * $p<0.05$ |
| 0.008 vs 0.010 | 0.9952 | 0.8999947 | insignificant |

## 10.4 DISCUSSION

There is no significant difference between any of the independent variable in the group without $H_2O_2$ and there was significant differences between some independent variables in the group with $H_2O_2$. This could possibly suggest the effect of neem only lasts about 24h which is why traditionally families take neem every day.

However, why there are still significant difference in the group with $H_2O_2$ cannot be explained using the current research. It can also be seen that it showed more significant differences then after 24h. This could be due to the lower concentrations losing their effects quite readily causing while the higher concentrations still showed effects after 48h. Hence, the difference between lower concentrations (0.002 and 0.004 g/cm$^3$) and the higher concentrations (0.006-0.010g/cm$^3$) would be higher and enough to produce significantly different results.