TED ANKARA COLLEGE FOUNDATION

PRIVATE HIGH SCHOOL

Investigating the effects of changing concentrations of Hydrogen Peroxide on DNAs of lentil (*Lens culnaris*) and chickpea *(Cicer arietinum)*plants.

BIOLOGY EXTENDED ESSAY

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Candidate Number: 1129-0009

Word Count: 3704

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Abstract

Strong chemical agents have been a part of our lives for quite some time; they have made many contributions in various fields, and are still being used frequently. However such common chemicals, like the ones that are being used in our houses for cleaning, can also bring harm to us and our environment. Aside from being used in industrial and domestic fields, such chemicals are also used in agricultural fields as, for example, pesticides. It is possible for these pesticides to harm plants, physically and even have mutagenic effects on it.

Considering these factors, this study concentrated on Hydrogen Peroxide (H_2O_2) which can be used in pesticides along with its usage as cleansing agent. Due to its nature, it is highly possible for H_2O_2 to bring harm to plant phsically and even affect its DNA, when present in excessive concentrations. The aim of this study was to investigate what sort of effect does different concentrations of H_2O_2 have on different plants, that being *Lens culnaris* and *Cicer arietinum.*

It was found that as H_2O_2 concentration was increased, damage done on plant DNA also increased, which was examined by the comet like formation of damaged DNA fragments. As the concentration increased, more fragments from the cell nucleus shattered and the comet got longer. The maximum amount of damage was done by the highest concentration 36%, after 15 minutes of exposure to H_2O_2. Overall the data obtained from the examination of comets support the hypothesis that H_2O_2 would have a similar harmful effect on the plants, regardless of their species.

Word count: 258

Key words: DNA Comet Assay Method, Hydrogen Peroxide (H_2O_2), lentil (*Lens culnaris),* chickpea *(Cicer arietinum),* pesticide.

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Introduction

I decided the topic which I will focus on this extended essay, by working my way up from "the general effects of Hydrogen Peroxide (H_2O_2)", to "harmful effects of H_2O_2 particularly on human tissue" and finally to "harmful effects of H_2O_2 particularly on plant tissue".

To elaborate this process: One particular thing that made me interested in studying H_2O_2 was its characteristic of being both harmful and helpful for different purposes. Being one of the most powerful oxidizers known [1], Hydrogen Peroxide (H_2O_2) has numerous applications in different fields and also has been a huge part of our daily life. From what I had known, H_2O_2 was a very powerful chemical that was equally harmful to human tissue and organs. However it had never occured to me that it was being used for its positive effects as well, which led my attention to its daily usage. Today, in addition to its common usage as consumer products (detergents), hydrogen peroxide is used in many daily tasks such as bleaching textiles and paper products[1], as well as processing or manufacturing foods, and minerals[1].

With what I had learned from my research, I concluded that despite it's benefits regarding health, environmental and industrial issues, it was evident that Hydrogen Peroxide also had its negative aspects in certain conditions. This lead me to carry out my research further to analyze its effects on specifically human tissue. In several articles it is stated that H_2O_2 is present in human body as a basic requirement [2] and also we are exposed to it via external factors on a regular basis: we exhale it, excrete it and take it in from diet [3]. Because of our constant exposure, there are systems and reactions -such as metal ion sequenstration- [4] that prevent the toxicity of those small amounts of H_2O_2 in our bodies. However if those mechanisms were to fail in some way, that can produce devastating tissue damages[4]. As a result, high concentrations of H_2O_2 can cause irriations, corrosions on tissue which may turn out to be fatal in some cases[5] and also has a potential to be mutagenic in vitro. In addition, highly reactive molecules called free radicals in H_2O_2 can cause tissue damage by reacting with fatty acids in cellular membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins. [6]

[1] h2o2.com Treatment Applications Using Hydrogen Peroxide, Hydrogen Peroxide Overview- "Hydrogen Peroxide (H_2O_2) is a Powerful Oxidizer"[1]

[2] "Hydrogen Peroxide - Curse or Cure?" Mountain House Publishing *(*Dr. David G. Williams) http://www.jaoa.org/content/105/3/145.abstract

[3] Halliwell, B. and Gutteridge, J.M.C. (1999) Free Radicals in Biology and Medicine, 3rd edn., Clarendon Press, Oxford.

[4] Halliwell, B. and Gutteridge, J.M.C. (1999) Free Radicals in Biology and Medicine, 3rd edn., Clarendon Press, Oxford.

[5] "Hydrogen Peroxide Toxicological Overview" Public Health England

[6] "Free radical tissue damage: protective role of antioxidant nutrients." (L J Machlin and , A Bendich

As my research about the harmful effects of H_2O_2 on human tissue progressed, I recalled that while searching the daily usage of H_2O_2, I had read that plants were also exposed to this material, particularly via pesticides. H_2O_2 is known as a common disinfectant; however, the Environmental Protection Agency (EPA) approved that Hydrogen peroxide is used as an ingredient in pesticides. During my research, I came across with several articles stating the positive effects of this material - specifically- on germination in which it was used to break dormancy by blocking Absisic Acid (ABA) and also to shorten the time required for germination [7]. However it was also stated that, even though it helped germination, the toxicity in the chemical eventually kills the plant [8].

Eventually my previous researches made me wonder whether H_2O_2 has similar harmful effects on plant tissue and DNA. The reason for me to focus on the possible damage on plant DNA was the considerable damage in human DNA and observing whether the effects were the same with a plant's DNA. Another reason, is the application of the DNA Commet Assay Method, in which the damage can be tested, determined and observed very clearly and accurately.

In order to observe the changes in DNA structure and evaluate regarding their usage in our daily life, I chose common plants lentil (*Lens culinaris*) and chickpea *(Cicer arietinum*). The experiment I will perform will be carried out by putting these species' samples into different concentrations of H_2O_2 solution (4.5%, 9%, 18% and 36 %) and observing the possible damage that is the forming of a comet like structure due to the shattered DNA fragments.

Consequently, this paper will focus on the question: What are the effects of different concentrations of Hydrogen Peroxide (H_2O_2) (4.5%, 9%, 18%, 36%) on the DNA structure of lentil (*Lens culnaris*) and chickpea *(Cicer arietinum*) plants, in which the damage is determined by DNA Comet Assay Method? as well explain and discuss how the experiment was planned and done along with the examination of the results and analyzing the consequences.

Word Count: 732

[7] "Effects of hydrogen peroxide on the germination and early seedling growth of barley under NaCl and high temperature stresses" EurAsian Journal of BioSciences (Cavusoglu K, Kabar K ; 2010)

[8] "The Effect of Hydrogen Peroxide on the Rooting of Plant Cuttings andSeed Germination"

Hypothesis

Evidences propose that, H_2O_2 therapy was proved to be successful in many non-life threathening situations [1] however it was also stated that; intake of high concentrations of H_2O_2 may cause burns in the mouth, throat, bleeding in the stomach, inflammation of the intestines. Another statement is that "An overuse of H_2O_2 can cause damage to DNA cells, and prevent them from replicating, hence leading to premature aging." Moreover, in recent studies it was shown that "H_2O_2, used as an antiseptic to treat wounds, actually obstructs scarless healing, since it destroys the newly formed cells" [1].Regarding H_2O_2's applications in environmental issues, Dani J. Barrington and Anas Ghadouani's study of "Application of Hydrogen Peroxide for the Removal of Toxic Cyanobacteria and Other Phytoplankton from Wastewater"[2] can be given as an example; in which they had proposed an application of H_2O_2 to induce cyanobacterial cell death. Although H_2O_2 is being used for cleaning toxic waste, it is stil evident that it is indeed a substance powerful enough to induce fatal cellular damage.

Assuming H_2O_2 is used as pesticide, while considering the facts above, even though it is used as a cleansing agent, there is rather high probability that some amount of H_2O_2 residue will remain on the nearby area and possibly on the plants and damage their tissues. Considering the very destructive effects of high concentrations of H_2O_2 on human tissue, it is likely to assume that high concentrations will also effect plants in a similar way. Thus, it can be hypothesized that different concentrations of H_2O_2 will have a harmful effect on the DNAs of lentil (*Lens culinaris*) and chickpea *(Cicer arietinum*). It is expected that gradually increasing concentrations of H_2O_2 will also increase the damage in the DNA of these plants.

Word Count: 291

[1] "Hydrogen Peroxide (H_2O_2) Benefits, Side Effects, Reviews and Facts" (www.dheaguide.info/hydrogen-peroxide/)

[2] "Application of Hydrogen Peroxide for the Removal of Toxic Cyanobacteria and Other Phytoplankton from Wastewater"

Method Development and Planning

After deciding to study the possible effects of H_2O_2 on DNA structure of plants, I visited my father's collagues, who have majored in biology, to hear their opinions and possible advices on the subject. I was discussing the laboratory conditions and whether I could use the laboratory under supervision and also, struggling to choose a proper method at the same time, since there was many applications and methods regarding the examination of DNA. As I was determined to conduct an experiment under the DNA Fingerprinting subject, one of my father's collegues proposed a more challenging idea and introduced me to the Comet Assay Method also known as Single-Cell Gel Electrophoresis, which was a much more efficient and very quicker way compared to DNA Fingerprinting Method.

I was told that this method was very useful and popular in many fields such as: Determination of the irradiated nutrients, toxicology genetics, diagnosis of genetic diseases, biomonitoring and the studies of damaging and repairing of the DNA.[1] As my father's collegues continued their explanation, I learned that they carried out an experiment with Comet Assay Method several years ago, to examine the effects of gamma radiation on the DNA samples taken from quail meat.[2]

After hearing that I wondered if the same method could be applied to my own research, so I asked if it was possible to determine the effects of H_2O_2 in plants with Comet Assay. In return, I was told that just like severe radiation exposure, several chemical agents could also cause a similar damage on the structure of DNA and was happy to know that the method was more than suitable to investigate the effects.

After deciding on the topic and the method I did a further research and tried to learn more about the Comet Assay. Basically, the Comet Assay Method is the examination of DNA damage on a single cell level which can be performed, in a very sensitive manner, using single-cell gel electrophoresis (SCGE) [3]. During this process, lams are coated with extracted DNA fragments and agarose gel and put into electrophoresis. Under the influence of an electric field fragments migrate from the cell nucleus (Cathode) to Anode [1], which results in a characteristic comet-like shape: the assay is thus also commonly known as the 'Comet Assay'.

DNA fragments that have lower molecular weight, migrate further from the cell nucleus in the direction of Anode. After electrophoresis, fragments are then dyed and identified using fluorescent markers and

thus, the information on the cellular damage can be obtained from the shape of the comet 'tail'. This is formed to one side of each cell nucleus, immobilised in the gel, with the cell nucleus commonly referred to as the comet 'head'.[3]

For the experiment, it was necessary for me to choose plants, or particularly seeds, that were easy to obtain and used commonly in our daily lives. Therefore, I had initially thought of using seeds such as bean, pea and chickpea. A noteworthy point is, although many of those seeds are available in almost anywhere, it was highly important that they should be dryed or unrefined (i.e. not freezed or boiled). Physical processes, even such simple ones like boiling or freezing could harm the seed's DNA. While the damage might have not been catastrophic, it could still affect the result so I had to re-modify the design of my experiment to find dry seeds that were neither frozen nor boiled. In the end, instead of bean and pea I used lentil and chickpea that had been readily available in the laboratory.

The hydrogen peroxide solution that was used in the experiment had the maximum concentration of 35-36%, which is the concentration limit that for the manufactured hydrogen peroxide. To provide a constant decrease in H_2O_2 concentration, we chose to halve the percent concentration to 18%, 9% 4.5% and finallly the Control group. The reason for us to have a Control group, meaning no H_2O_2, for both lentils and chickpea was to have a guide to make a good comparison and to achieve precise results. No H_2O_2 means there will be no chemical damage to DNA, and with a normal DNA sample in the hand we would be able to analyse the damage clearly.

In order to reduce the possible errors and and to properly judge the results, the samples were prepared with two parallels, meaning there were two lamella, meaning two parallels, for each concentration (i.e. two lamella for the chickpea sample that was placed in 36% hydrogen peroxide solution). Again, to obtain reliable results, there would be five seeds for each concentration (i.e five lentils to be put in 36% hydrogen peroxide solution) and therefore on each lamella there would be DNA from alt least five lentil seeds.

As for the other variables, the room temperature was held constant at 22°C because DNA samples could've easily been harmed because of the temperature. Time durations were of course highly significant, so all samples were held 15 minutes in H_2O_2 solution, 20 minutes in lysis solution, and 2 minutes in electrophoresis.The temperature of the agarose gel was kept at 42°C or below, and the

room conditions that could have any effect on DNA samples were constant since the experiment was conducted in the same room in one day.

One of greatest challenges I faced was, since a Comet Assay experiment with H_2O_2 on lentil and chickpea was carried out for the first time; we were not sure how long the seeds would have to wait in the H_2O_2 solution. So, we had to estimate what would be the optimum time taken for the damage to occur. We tried to calculate and compare the information from the previous experiments. To extract the DNA for electrophoresis, we decided using lysis solution to quickly destroy the membranes. Again, since this experiment was carried out for the first time, the same problem also arised for the usage of lysis solution. It is known that since an animal doesn't have a very thick cell and core membrane, it is very easy to break it and extract the DNA fragments and also, the process would only take about three or four minutes. However since the plant cells have a cellulose cell wall it needed more time in lysis, but then again not too much to tear apart all of the DNA fragments.

In the end, after the migration of fragments from cell nucleus to anode diraction, the samples were examined via Digital Imaging System[1] that help us interpret the image based on pixels. The system includes microscope and Bs200ProP Software with additional geometric calibrations[1], which determmines how the images obtained from the microscope will be processed. [1] With the necessary software, images are converted into numerical datas based on such parameters as the following[1]:

Tail Length, Tail Moment, % DNA in tail, etc., and with calculations from the RGB color pixels, datas are provided for these parameters.

Word Count: 1153

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[1] "DNA Komet Analiz Yöntemi" (Nizamettin Yazıcı, Turkish Atomic Energy Agency, Saraykoy Nuclear Research and Training Center)

[2] "Detection of irradiated quail meat by using DNA comet assay and evaluation of comets by image analysis" (Yakup Erel, Nizamettin Yazici, Sumer Ozvatan, Demet Ercin, Nurcan Cetinkaya; 2009)

[3] "A High Sensitivity, High Throughput, Automated Single-Cell Gel Electrophoresis

('Comet') DNA Damage Assay" (B. Vojnovic, P.R. Barber, P. Johnston, H.C. Gregory

B. Marples and M.C. Joiner, R.J. Locke; 2013

Material List

1. 10 beakers
2. 25 grams of Chickpea
3. 25 grams of Lentil
4. 36% H_2O_2 solution
5. Chronometer
6. Phosphate Buffered Saline (PBS) solution (pH 7.4)
7. Magnetic stirrer
8. 20 lamellas
9. Water bath
10. Micropipette
11. Power source (i.e. 0-100 V)
12. Olympus BX51 Fluorescent Microscope
13. BAB Bs200ProP Digital Imaging System
14. Agarose gel at 42°C
15. Horizontal Electrophoresis System
16. Tris(hydroxymethyl)aminomethane (TBE) solution
17. Lysis (buffer) solution
18. Muller

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Method

1. Prepare 10 beakers, separate 5 for chickpeas and 5 for lentil.
2. Prepare 5 grams of chickpea for each 5 beakers (making 25 grams of chickpea in total).
3. Prepare 5 grams of lentils for each 5 beakers (25 grams of lentils in total).
4. Label each beaker for different concentrations of H_2O_2.

(“36% C”, “9% C”, “4.5% C”, “Control C” for chikpeas and “36% L”, “9% L”, “4.5% L”, “Control L” for lentils)

5. Put 5 grams of lentils and chickpeas into labelled beakers.
6. Put 10 ml of of 36% H_2O_2 into beakers labelled “36% C” and “36% L”
7. Dilute 20 ml of 36% H_2O_2 by half, to 18% by adding water and separate the diluted solution into 10 ml for “18% C” and “18% L”
8. Repeat step 7 for 9% H_2O_2 and 4.5% H_2O_2 and put the solutions into labelled beakers, 10 ml each.
9. Keep the seeds 15 minutes in H_2O_2 solutions.

<u>Comet Assay</u>

After 15 minutes pour the H_2O_2 solutions away, keeping the seeds inside the beakers

Transfer the seeds into separate cups and crush them lightly with muller, then transfer them back into initial beakers.

Proceed with the DNA Extraction procedure: bathe them in PBS solution and then put hem in a Magnetic Stirrer to extract their DNA.

Label the lamellas and prepare them for Electrophoresis by coating them with Agarose gel.

Mix the DNA extracts with Agarose gel and coat the lamellas with it and after they are dried put them in Lysis solution. After some time tke the samples out of lysis solution and bath them in TBE solution. Then put them in electrophoresis tank for 2 minutes. After taking the samples out, put them in pure water to get rid of the residue. Finally dye the lamellas with Ethidium Bromide solution andexamine them under Fluorescent microscope.

Word Count: 307

Processing the Data

Table 1: Raw Data For Chickpea

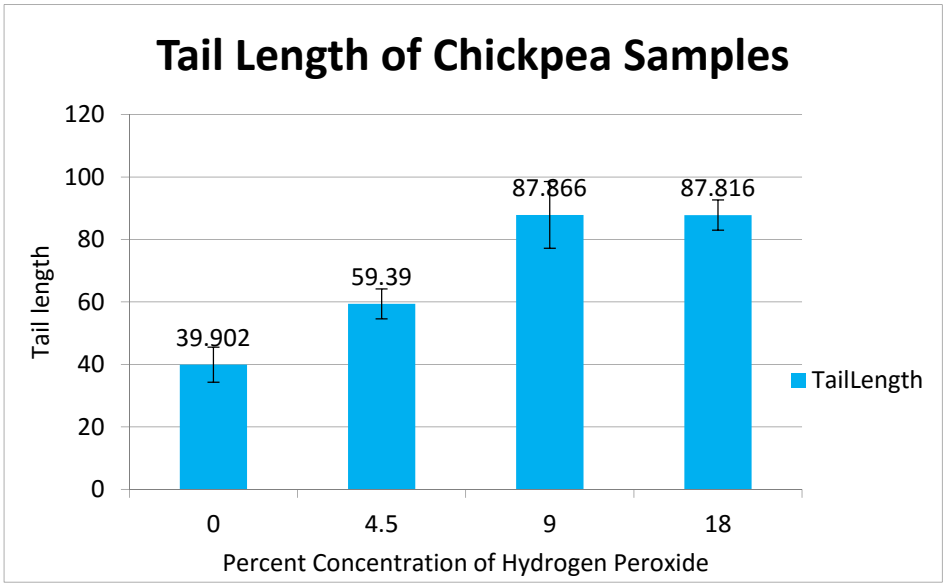
Sample Numbers	Percent Concentration of Hydrogen Peroxide(%)	Comet Area	Comet Length	DNA Head	Head Length	Head Area	Tail Length	Tail Moment
1	0	2328,5	54,06	34,82	19,04	819,89	35,03	22,83
2	0	2760,19	66,24	40,09	25,89	1078,7	40,36	24,18
3	0	4196,43	82,23	41,67	36,55	1865,08	45,69	26,65
4	0	2887,45	61,68	44,09	25,89	1212,02	35,79	20,01
5	0	3778,64	76,14	44,52	33,5	1662,6	42,64	23,66
1	4,5	4170,77	89,09	40,57	29,7	1390,26	59,39	35,3
2	4,5	4277,71	91,37	38,31	28,93	1354,61	62,44	38,52
3	4,5	4063,83	86,8	35,97	31,22	1461,55	55,58	35,59
4	4,5	4242,06	90,61	46,39	26,65	1247,67	63,96	34,29
5	4,5	3921,23	83,76	30,58	28,17	1318,96	55,58	38,59
1	9	14326,57	141,12	44,71	43,65	4431,96	97,46	53,88
2	9	5090,48	103,55	27,09	27,41	1347,48	76,14	55,52
3	9	5881,85	125,63	33,69	37,31	1746,73	88,32	58,57
4	9	5417,72	113,45	33,75	30,46	1454,42	82,99	54,98
5	9	6278,61	130,2	39,74	35,79	1725,7	94,42	56,89
1	18	4990,66	106,6	16,51	19,8	926,84	86,8	72,47
2	18	5061,96	108,12	18,49	23,6	1105,08	84,52	68,89
3	18	5204,55	111,17	20,68	21,32	998,13	89,85	71,27
4	18	5632,32	120,3	23,59	27,41	1283,31	92,89	70,98
5	18	5646,58	109,64	21,99	22,08	1137,16	87,56	68,31

Table 1. Raw Data from the Image Analysis System, Illustrate the raw datas obtained from the pixel based analysis of the shattered DNA fragments on lamellas. (ScaleUnit Micron µm). Datas also represent the Comet Assay Parameters, which have a high importance regarding the examination and evaluation of the damage.

Table 2: Processed Data For Chickpea:

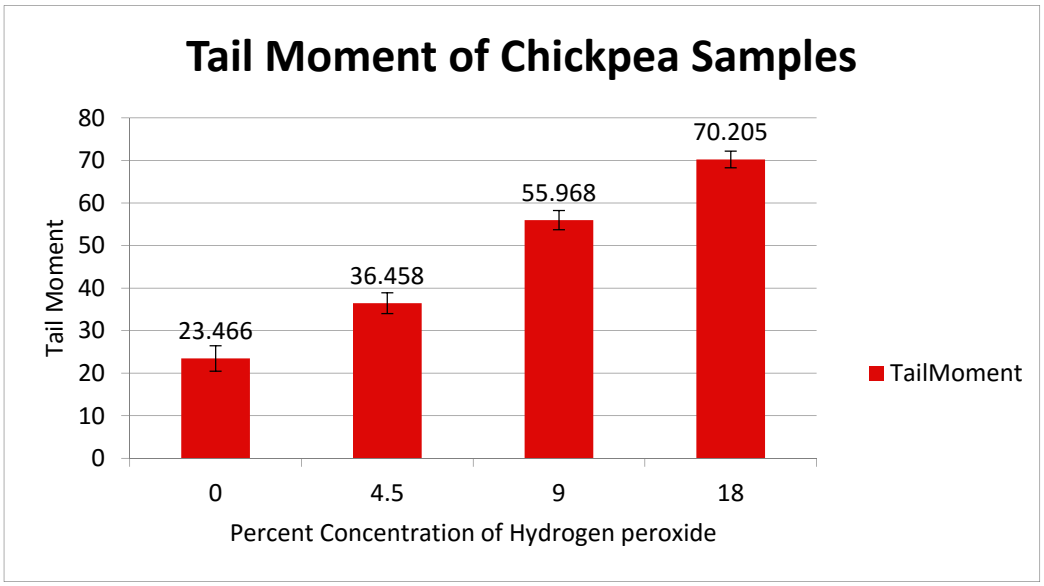
MEAN VALUES				
Tail Length	STANDARD DEVIATION/EROR	Percent Cocentration of Hydrogen Peroxide(%)	Tail Moment	STANDARD DEVIATION/EROR
39,902	4.52/ 0.90	0	23,466	2.40/ 0.48
59,39	3.85/ 0.77	4,5	36,458	1.97/ 0.39
87,866	8.61/ 1.72	9	55,968	1.81/ 0.36
87,816	3.91/ 0.56	18	70,205	1.58/ 0.23

Table 2. Shows the processed data for the Comet Assay of Chickpea. The values show the mean measurements of the parameters as well as the Standard deviation and error values.



(Graph 1) Tail Length of the Chickpea Samples

Depicts the gradual change in tail length, which also determines how the increasing concentations of Hydrogen peroxide, increase the damage in DNA, here in this case, in terms of tail length.



(Graph 2) Tail Moment of Chickpea Samples

Also depicts the effects of Hydrogen peroxide on DNA with error bars of 95% Confidence Interval via the increase in the tail moment.

Table 3: Raw Data For Lentil

Sample Numbers	Percent Concentration of Hydrogen Peroxide(%)	Comet Area	Comet Length	DNA Head	Head Length	Head Area	Tail Length	Tail Moment
1	0	1853,67	39,59	32,39	12,18	570,36	27,41	18,53
2	0	1834,42	47,21	37,3	17,51	680,51	29,7	18,62
3	0	1156,05	35,79	35,02	9,9	319,76	25,89	16,82
4	0	992,43	36,55	39,91	11,42	310,13	25,13	15,1
5	0	3814,29	81,47	39,67	30,46	1425,9	51,02	30,78
1	4,5	3956,88	84,52	38,48	28,93	1354,61	55,58	34,19
2	4,5	3636,05	77,67	33,35	24,37	1140,72	53,3	35,53
3	4,5	3743	79,95	39,4	27,41	1283,31	52,54	31,84
4	4,5	3707,35	79,19	38,03	28,17	1318,96	51,02	31,61
5	4,5	4313,36	92,13	30,77	25,13	1176,37	67,01	46,39
1	9	4242,06	90,61	27,27	22,08	1033,78	68,53	49,84
2	9	4277,71	91,37	26,35	25,13	1176,37	66,24	48,79
3	9	4491,6	95,94	31,97	27,41	1283,31	68,53	46,62
4	9	4562,89	97,46	26,71	26,65	1247,67	70,81	51,9
5	9	4063,83	86,8	28,98	25,13	1176,37	61,68	43,8
1	18	5846,2	124,87	33,9	40,36	1889,32	84,52	55,87
2	18	4598,54	98,22	24,58	23,6	1105,08	74,62	56,27
3	18	4420,3	94,42	27,3	25,13	1176,37	69,29	50,38
4	18	5382,79	114,97	33,38	37,31	1746,73	77,67	51,74
5	18	5561,02	118,78	40,4	37,31	1746,73	81,47	48,55

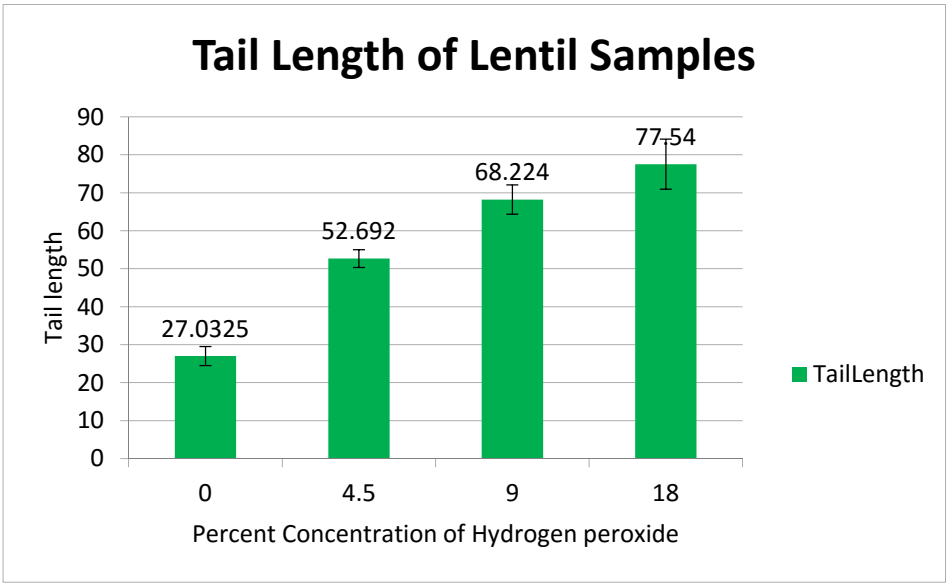
Table 3. Shows the raw datas resulting from the Comet Assay analysis of lentil DNA Fragments. With these results from the Digital Imaging System, damage made to Lentil's DNA can easily be evaluated considring the comet assay parameters.

Table 4: Processed Data For Lentil

MEAN VALUES				
Tail Length	STANDARD DEVIATION/EROR	Percent Cocentration of Hydrogen Peroxide(%)	Tail Moment	STANDARD DEVIATION/EROR
27,0325	2.02/ 0.50	0	17,2675	1.67/ 0.42
52,692	1.89/ 0.38	4,5	32,79	1.99/ 0.40
68,224	3.10/ 0.52	9	48,708	2.87/ 0.48
77,54	5.31/ 0.88	18	52,52333	3.04/ 0.51

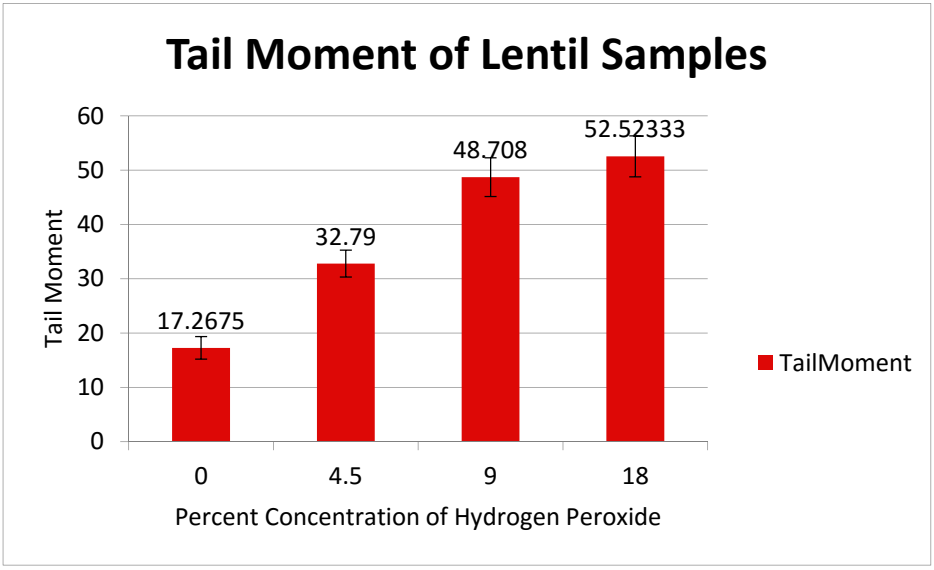
Table 4. Illustrates the mean values of the necessary Comet Assay parameters.

***Notice that the concentration 36% is not included in the tables of both lentil and chickpea because of the high damage of Hydrogen peroxide on the plant DNA. 36% concentration shattered the DNA so much that no clear shape could be obtained from it in both chicpea and lentil, so nothing could be analysed from the images.*



(Graph 3) Tail Length of the Lentil Samples

Depicts the gradual change in tail length with error bars of 95% Confidence Interval, which also dtermines how the increasing concentations of Hydrogen peroxide, increase the damage in DNA, here in this case, interms of tail length.



(Graph 4) Tail Moment of Lentil Samples

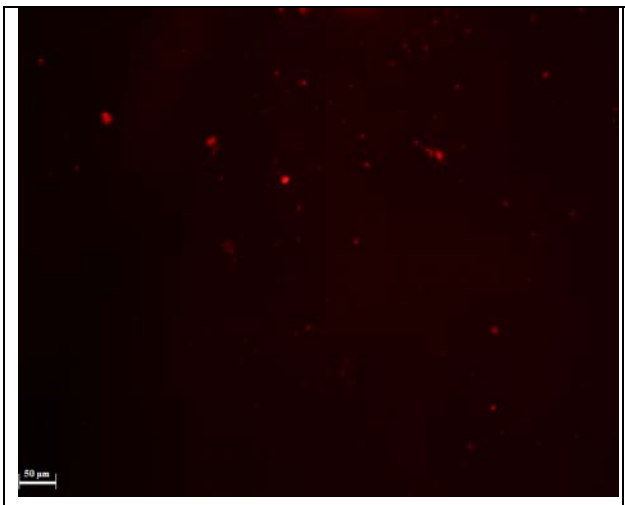
Also depicts the effects of Hydrogen peroxide on DNA clearly with the change in the tail moment with error bars of 95% Confidence Interval.

(Figure. 1)

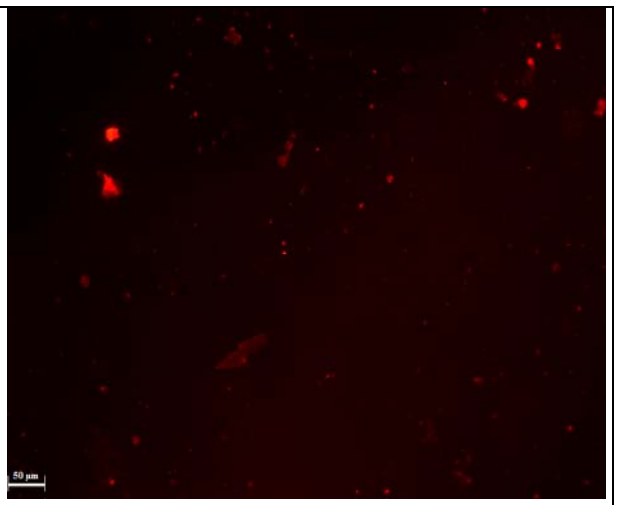
Tail Length t-Test: Two-Sample Assuming Unequal Variances			Tail Moment t-Test: Two-Sample Assuming Unequal Variances		
	Tail Length Chickpea	*Tail Length Lentil*		*Tail Moment Chickpea*	*Tail Moment Lentil*
Mean	68,7435	56,37213	Mean	46,52425	37,82221
Variance	549,5834	487,6343	Variance	427,6582	260,8142
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6	
t Stat	0,768268		t Stat	0,663297	
P(T<=t) one-tail	0,235737		P(T<=t) one-tail	0,265891	
t Critical one-tail	1,94318		t Critical one-tail	1,94318	
P(T<=t) two-tail	0,471473		P(T<=t) two-tail	0,531783	
t Critical two-tail	2,446912		t Critical two-tail	2,446912	

Figure 1. Shows the results of an Independent T-Test between data groups of Lentil and Chickpea(mean values of Tail Length and Tail Moment inTable 2 and Table 4).

Images of DNA Samples Under Microscope



Chickpea 36%



Lentil 36%

(Figure 2.)

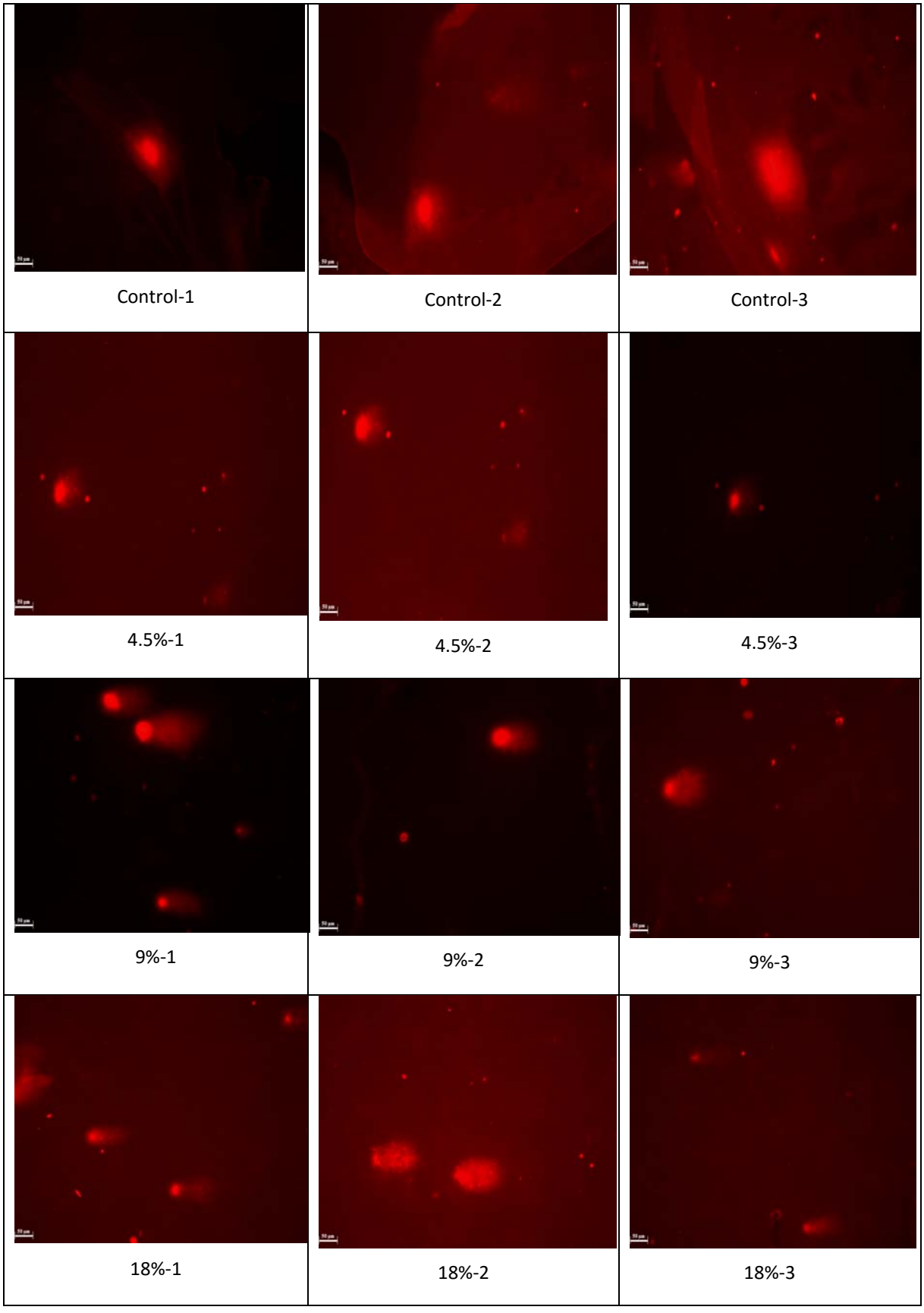


Figure 3. Images of migrated DNA fragments from Chickpea samples.

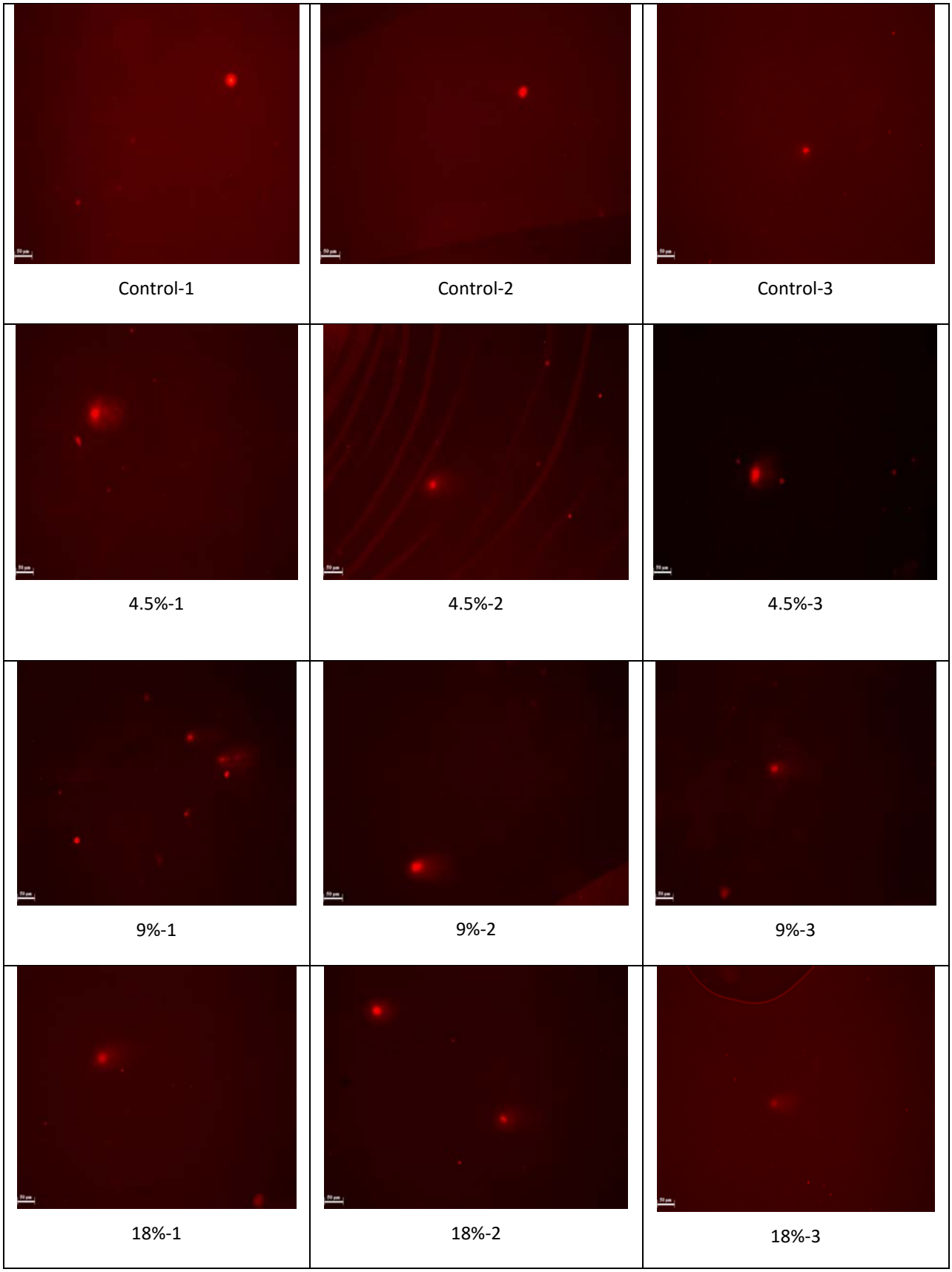


Figure 4. Images of migrated DNA fragments from Lentil samples.

Figure 2, Figure 3 and Figure 4 all depict the images of DNA fragments that had been shattered by Hydrogen Peroxide and later migrated on the lamella via Electrophoresis, creating a comet like shape. The images were taken with Olympus BX51 Fluorescent Microscope in the scale of 50 µm, after the lamellas and also the DNA fragments were dyed with Ethidium Bromide (fluorescent dye). Notice that fragments has started to closely resemble a comet like shape more with the increasing concentrations of Hydrogen Peroxide and in the highest concentration of Hydrogen Peroxide (36%) there is almost nothing but very small and scattered DNA fragments that are no longer in any shape, which shows the extent of the damage.

Conclusion

H_2O_2 is said to be a powerful oxidizing agent, that has been in our daily lives on a regular basis. And among it's positive applications such as cleansing agents, it is still a chemical that can be very harmful and may have serious effects on the living organisms, be it gradual or immediate (see introduction). A very recent example, that may show the vulnerability of human tissue against H_2O_2 has taken place during this experiment. One of my supervisor's finger was exposed to 36% H_2O_2, and the skin that had contact with it, immediately changed colour and became paler. With such a powerful chemical, it is inevitable to think further of the environmental consequences of its usage.

It would of course be interesting to study why the living tissue is vulnerable against strong chemicals (or rather more specifically, H_2O_2) on a cellular basis, before addressing a solution for that problem. Aside from external exposure, the intake of H_2O_2 is a regular process in our bodies, as it is required. However if mechanisms that prevent the toxicity of H_2O_2 were to fail in some way, that can produce devastating tissue damages[1]. As a result, be it from external exposure or biological intake, high concentrations of H_2O_2 can cause tissue damages such as irritations, corrosions, etc. Thus, the question which followed this was whether H_2O_2 had a similar effect on plants as well.

And as the experiment proved that there is indeed a damage in *Lens culnaris and Cicer arietinum's* DNA made by Hydrogen Peroxide (H_2O_2). It was suggested that, with increasing percent concentration of H_2O_2, the damage on plant's DNA will also increase and with the results, the hypothesis was confirmed. Even though there are some difference in recorded data between *L.culnaris* and *C.arietinum*, the harm of H_2O_2 proves to be very similar on plant tissue. In fact, the damage was proved to be severe especially on higher concentrations up to point that in 36% DNA fragments shattered in such a way that there was no visible comet formation on lamellas. In addition, to reach a general statement of confirmation and also in order to analyze and evaluate other data thoroughly, with the help of computer image analysis program the comets were analysed and evaluated in many parameters and in the end sufficent data were compiled in tables and graphs.

The results and further statistical analysis as shown in tables and graphs, support the hypothesis and the Independent T-Tests carried out between the data groups of the key parameters in assesing the damage(Tail Length and Tail Moment) shows that there is no meaningful difference between the data groups. This suggests that not only Hydrogen peroxide affects the Tail Moments and Tail Lengths of each plant, but also affects them wğth a relation to each other.

Previously mentioned factors might be the reason why such strong effects have been observed on plants. If we take a closer environmental approach, such severe damage to seeds can be alarming because even in low concentration (4.5%, 9%) there was a significant damage in DNA in terms of increasing tail formation and length (For example in 27.0325 µm in 0% and 77.5 µm in 36 % for Chickpea). This much physical and cellular damage as illustrated in Graphs above, suggests that the plant has been harmed and it can further can lose its ability germinate, or even if it germinates the damage may effect the next generations and can even lead to mutated offsprings. If pesticides that contain, such strong chemical agents are used, or released in the environment, in the long run, this can upset the balance of ecosystem and endanger the plant species.

The potential environmental hazards of such chemicals can reach and effect the humans themselves. Although they are in some cases beneficial, the effect of these chemicals have on the environment is too prominent to ignore. Strong chemicals are needed in order to destroy harmful organisms and to produce healthy products. In order not to disturb the environment while doing that, more researches are needed to be carried out to find out the exact extent of harm these chemicals are doing to nature and humans themselves and reduce the damage as much as possible.

Word Count: 704

[1] Halliwell, B. and Gutteridge, J.M.C. (1999) Free Radicals in Biology and Medicine, 3rd edn., Clarendon Press, Oxford.

Evaluation

In order to analyse how DNA was affected by H_2O_2 concentrations varying from 0% to 36%, the images recorded via fluorescent microspcope were transferred to a software and

as it is visible from the graphs, the damage grows steadily (displayed by Graphs 1, 2, 3 and 4) with constantly increasing concentrations of H_2O_2.

Although the results are clear and the data follows a steady increase, there are errors in the experiment that had been most likely caused by the DNA extraction process of the experiment. These errors can be reduced by repeating trials, and in this case, by using the second paralel of lamellas and include new data.

However not all errors can be eliminated by repeating trials. A noteworthy point is that, the errors could be excluded by changing the procedure slightly, because of the possibility of damaging the DNA furthermore by the lysis process. It is highly possible that the lysis process could have done futher harm to DNA during the melting of cell membrane to fully extract DNA. The DNA Comet Assay is an efficient method for extracting and analysing the DNA quickly. Therefore melting of cell membrane by lysis process is required to be carried out, so it would not be wise to exclude lysis to alter the procedure. However whatever possible damage done by lysis solution, can be reduced by shorthening the time that the samples spend waiting in the solution. Due to the fact that this experiment had not been carried out with H_2O_2 before, my supervisors at the lab had to decide for a suitable amount of time. If we had known a specific time for the lysis process, shorther time in lysis solution might have created better results with less error. One other source of error can be, although it is not highly possible, crushing of the seeds, although it is a physical damage, could have affected and increased the damage in DNA.

Despite the small amouts of error, the results are valid, as it indicates that like humans or other plant tissue is vulnerable such powerful chemicals. The fact that H_2O_2 can affect the DNA of seeds, and also change the course of plants' life up to a point where development and further germination of the seed might be affected from this damgage is supported by the experimental results. And thus, adds to accumilated evidence on the basis of which this statements was formulated, which ultimately leads to the consideration of the bigger picture, and the evaluation of the experiment with a global perspective. It is not possible to consider the effects of such chemical agents and and more specifically pesticides without a global approach which gives birth to an issue that needs careful consideration. As my experiment proved there are many effects of chemical agents on plants just like it is on humans, and them being used on agricultural products can return back to us and the world with harmful effects

multiplied by many times. So it is essential to take precautions before the usage of chemical agents like Hydrogen Peroxide damage us and our environment severely.

Word Count: 517

Appendix

Standart Comet Assay Method:

1. Extraction of DNA
2. Put the samples on Magnetic stirrer after bathing them Phosphate Buffered Saline (PBS) solution, to mix their DNA extracts with the solution.
3. After 15 minutes, stop the stirrer and transfer the solution that contains the extracted DNA, into tubes, leaving the sediment in the beaker. Leave the tubes in a refrigirator (or generally, cold atmosphere).
4. Coat the lamellas with agarose gel that had been kept at constant temperature in water bath. Leave them to dry.
5. Take the sample solutions out of the refrigirator. With different micropippettes respectivetly, pick a small amount of agarose gel and some DNA extract solution and mix them in a different tube.
6. Coat the lamellas after they have dried but this time, with that mixture of DNA extracts and agarose gel. Cover the lamellas with thin protective glass.
7. Putt the first group into lysis solution to prepare the samples for electrophoresis.
8. After keeping the samples in Lysis solution for 20 minutes, take them out and put them into TBE solution to stop the effects of Lysis solution.
9. After the bath in TBE solution, align the samples alongside in the electrophoresis tank. Set the power at 62 V and start the electrophoresis. Keep the lamellas in there for 2 minutes.
10. After putting lamellas of the first paralel together, put them into pure water to cleanse the lamellas from the residue minerals of electrophoresis. After the water bath, leave them to dry.
11. Prepare a solution of Ethidium Bromide (fluorescent dye) under the fume hood and after the samples are dry enough, submerge them into the Ethidium Bromide solution, while still under the fume hood. After the dying process, rinse them with towels.
12. Put the lamellas of the first parallel under fluorescent microscope, while reducing the light in the room by turning the lights off and closing the curtains. Transfer the images seen on the microscope to the computer and save it to later be analysed with BAB Bs200ProP programme.

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