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Extended essay cover

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Examination session (May or November)	May
Year	2012

Diploma Programme subject in which this extended essay is registered: BIOLOGY

(For an extended essay in the area of languages, state the language and whether it is group 1 or group 2.)

Title of the extended essay: EFFECT OF KINETIN (N6 FURFURY-LADENINE) ON THE LEAVES AGING AND BIO-SYNTHESIS OF CHLOROPHYLL (BARLEY)

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Effect of Kinetin (N6 furfuryladenine) on the leaves aging and biosynthesis of Chlorophyl

Session: May 2012

Subject of the Extended Essay: Biology

Word count: 3989

Abstract:

The investigation which I conducted aims at showing, what in fact is the effect of the growth regulator Kinetin on the leaves aging. By treating leaves with different concentrations of Kinetin (KN) I've tried to figure out what will be the Chlorophyll level of these leaves after a week. As evidence of Chlorophyll level serves the absorbance of the treated probes at 665nm, measured by using a spectrophotometer Selecta.

Barley leaves were used for this experiment. I've tested the effect of 5 Kinetin concentrations. The concentrations which I've prepared were: 1mg KN/ 500ml H_2O; 2 mg KN/ 500ml H_2O; 3mg KN/ 500ml H_2O; 4mg KN/ 500ml H_2O; 5mg KN/500ml H_2O

Cut leaves from 12 days old barley plants were placed in 18 different Petri dishes. Three Petri dishes, filled with barley leaves were used for each KN concentration. The first three of them were used for control probes, the other 15 Petri dishes were used for the leaves treated with specific concentration of KN. Several days after that, the leaf explants were homogenized into a mortar and the Chlorophyll absorbance of the extracts was measured by a spectrophotometer. The experiment was done five times. Reading the value of Chlorophyll absorbance, a dependency has been found between the concentration of Kinetin and the Chlorophyll concentration.

The results showed up that with the increasing of the concentration of Kinetin, the value of the Chlorophyll absorbance is increasing too.

The lower values of the uncertainties made through the trials made prove the high precision of experimental work and show a reliability of the experiment. Looking at these facts and assertions, a conclusion could be made that the highest concentration of Kinetin affects the leaf explants in a way that it delays their aging.

Table of Contents:

Acknowledgements 4

Research Question 5

Hypotheses 6

Introduction 7

Apparatus 8

Method 9

Results 10

Experimental Results *10*

Uncertainties *15*

Analysis and Evaluation 22

Analysis of data and graphs *22*

T-test analysis *25*

Analysis of the uncertainties *26*

Improvements 27

Conclusion 28

Bibliography 29

Appendix 30

Data results *22*

T-test *25*

Pictures *26*

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Research questions:

- Does growth regulator Kinetin really have some effect on the plants' aging?
- How does the growth regulator Kinetin, affect plant's aging?
- Are there obvious differences between the leaves treated with different concentrations of the growth regulator Kinetin and the leaves treated with distilled water?

Hypotheses:

Zero hypotheses Ho

- The growth regulator Kinetin doesn't affect the plants' aging.
- The Kinetin doesn't slow down the leaf aging of cut plant leaves as well as it doesn't affect the biosynthesis of Chlorophyll in them;
- Different concentrations of Kinetin do not affect differently the process of cut leaves aging neither the biosynthesis of Chlorophyll in them.

Alternative hypotheses H_A

- I predict that Kinetin will show its properties as growth regulator and it will affect the leaf aging;
- I predict that the Kinetin will slow down the aging of the cut plant leaves, which is indicated by high Chlorophyll concentration in them;
- I expect that the Chlorophyll level in the treated leaves will increase with the increase of Kinetin concentrations.

Introduction

This Extended Essay tries to explain the effect of different concentrations of Kinetin (KN), which is one of the most widespread growth hormone, on plants' aging. Leaves treatment took place by observing the results of this experiment.

Cytokinins are plant hormones which promote plant division (cytokinesis) in plant roots and shoots. They are involved in cell growth and differentiation, apical dominance, auxiliary bud growth and leaf senescence.

There are two types of cytokinins. The first of them is adenine-type cytokinins- kinetin, zeanin, 6-benzylaminopurine; most of them are synthesized in roots. The second type cytokinins are called phenylurea-type cytokinins- dyphenilurea and thydiazuron (TDZ). Phenylurea cytokinins cannot be found in plants. Cytokinins participate in local and long-distance signaling, with the same transport mechanism as purines and nucleosides. Usually cytokinins are transported in xylem.[1]

The functions of the cytokinins were firstly discovered by Folke Skoog in 1940. He discovered their effect using coconut milk. One of the cytokinins' functions is greening, which means that they promote the light induced formation of chlorophyll and conversion of **etioplasts** to chloroplasts, e.g. greening process.[2]

Kinetin was the first cytokinin discovered. It was named like that because of its ability to promote cytokinesis. [1] [3]

Growth regulators are phytohormones and they exist in different varieties - cytokinins, giberillins, auxins, ethylene, etc. Kinetin functions as an antioxidant. Therefore it can prevent oxidative damages caused by dangerously reactive molecules which can destroy cellular proteins and DNA. This is a reason why Kinetin is used as an additional substance to anti-aging skin care products.[5] In the Botany, Kinetin is found in parts of plants and yeast. The plant hormone is also used in the agriculture because it helps for preventing the degeneration demolition of leaves. Besides Kinetin is used as a seed stimulator, which affects the germination in dormant seeds. A Japan research found that when husked dormant seeds are immersed in a solution of distilled water and Kinetin, the seeds start to germinate.[4] [5] [6]

Apparatus

The materials and substances needed for this investigation are listed below:

- 10-12 days old barley plants grown in soil
- Solutions of Kinetin with different concentrations: 0,2mg/100 ml, 0,4mg/100 ml, 0,6 mg/100 ml, 0,8 mg/100 ml, 1,0 mg/100 ml
- Ethanol (80%)
- Distilled water
- Petri dishes
- Filter Paper
- Cotton
- Pipette (5 cm^3)
- Measuring cylinder of 10 ml, ±1 ml
- Centrifuge tubes
- Centrifuge Selecta
- Spectrophotometer Selecta, ±0,001

Picture 1: Petri dishes with leaf explants, treated with different concentrations of the growth regulator Kinetin (KN)

Method

The methodology which I used is designed to investigate the effect of the growth regulator Kinetin on the aging of cut plant leaves. The procedure could be divided into 3 parts:

❖ Barley is grown in vessels filled with soil. At the 12^{th} day after the seeds were sown, the leaves are cut. Leaves with similar form and color are chosen. A piece 2 cm long is cut from the middle of every leaf.

❖ Eighteen Petri dishes are taken. After putting cotton and filter paper in each Petri dish every three of them are moistened with $10cm^3$ of different concentration of Kinetin, as the first three dishes are moistened with distilled water.

❖ Ten pieces of the cut leaves are placed in every Petri dish, where they stay for a period of 8 days. After the 8^{th} day the leaves are ready for investigation.

❖ Three of the cut leaves are taken from every Petri dish and put in different mortars. In each of the mortars, a solution of 80% ethanol which volume should be from 3 to 5 ml and a small amount of SiO_2 and $CaCO_3$ are added. The leaves are grinded (suspended).

❖ After they are finely ground, the suspension is put into a centrifuge tube and then-in the centrifuge, where it is centrifuged for 5 minutes, at 3000 revolutions per minute.

❖ After the centrifuge tubes are brought out, the solution above the precipitate on the bottom is pour out and then it is put into tubes for spectrophotometric analysis. The chlorophyll absorbance of the extract is measured at wavelength of 665 nm by using a Spectrophotometer Selecta. An 80% ethanol is placed as a control.

❖ After reading the Chlorophyll absorbance of each investigated probe, the results are written in the notebook.

❖ The method is done for each of the Petri dishes separately and when all of them are investigated, the trial is done.

In order to achieve more reliable experimental results the method was repeated five times so five trials were done.

Results

Experimental Results:

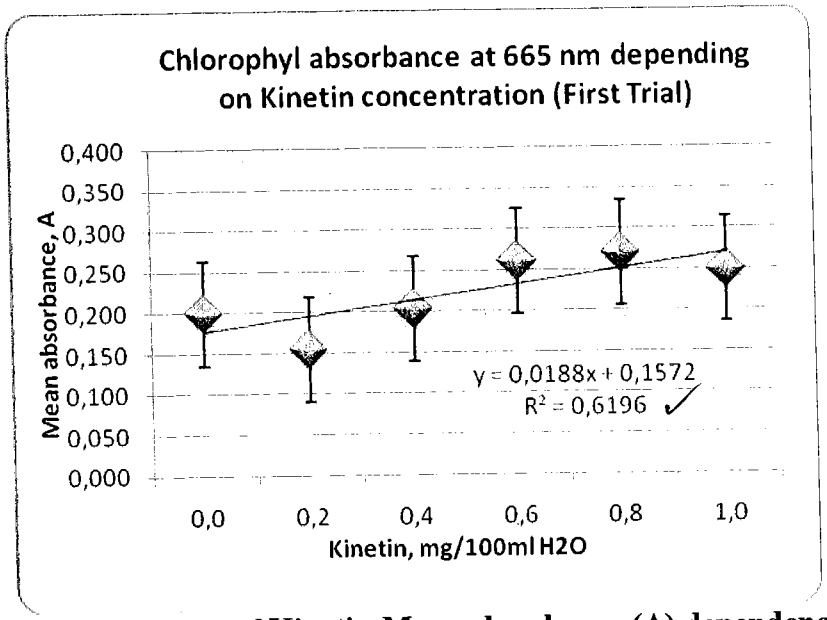
All the results from the experiment are shown in the Appendix at the end of this Extended Essay.

FIRST TRIAL

	ABSORBANCE OF THE SUSPENDED EXPLANTS AT 665nm					
	Control Probe, d H20	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
Mean $\bar{X}$	0,199	0,155	0,203	0,261	0,270	0,250
Standard deviation		0,027	0,064	0,023	0,015	0,035

Table 1: Barley leaf explants, treated with different concentrations of Kin (First trial)

In the Table 1 are represented the experimental results of the First trial of the experiment. The Mean values ($\bar{X}$) and the Standard deviation (SD) for each concentration of Kinetin are calculated by using Excel.



Graph 1: Concentration of Kinetin-Mean absorbance (A) dependence.

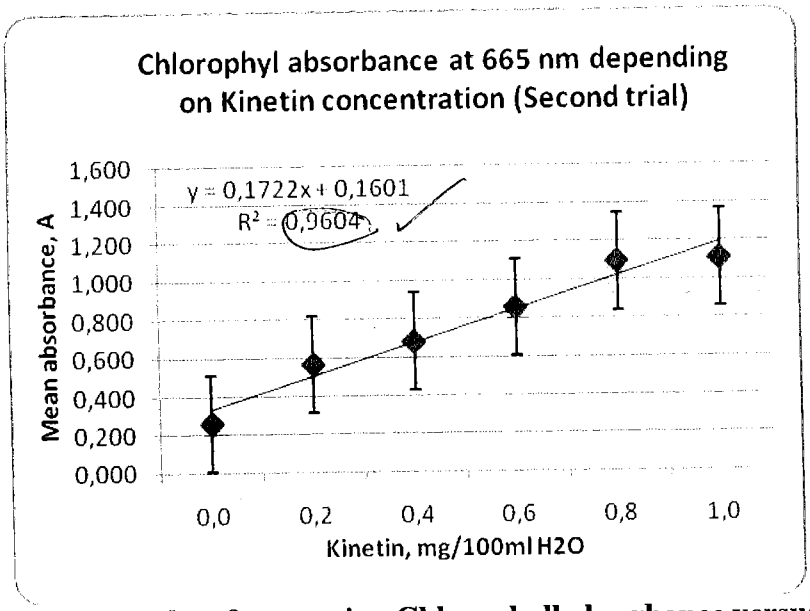
The Graph 1 represents the relationship between concentration of the Kinetin solutions and the Mean absorbance (A) of the control probe and the treated probes at 665 nm.

SECOND TRIAL

	ABSORBANCE OF THE SUSPENDED EXPLANTS AT 665nm					
	Control Probe, d H20	0,2 mg/100 ml KN	0,4 mg/100 ml KN	0,6 mg/100 ml KN	0,8 mg/100 ml KN	1,0 mg/100 ml KN
Mean ($\bar{X}$)	0,259	0,566	0,685	0,859	0,977	1,095
Standard deviation	0,135	0,129	0,149	0,121	0,254	0,205

Table 2: Barley leaf explants, treated with different concentrations of Kinetin (Second trial)

In the Table 2 are represented the experimental results of the second trial of the experiment.



Graph 2: Experimental results of measuring Chlorophyll absorbance versus different concentrations of Kinetin (Second Trial)

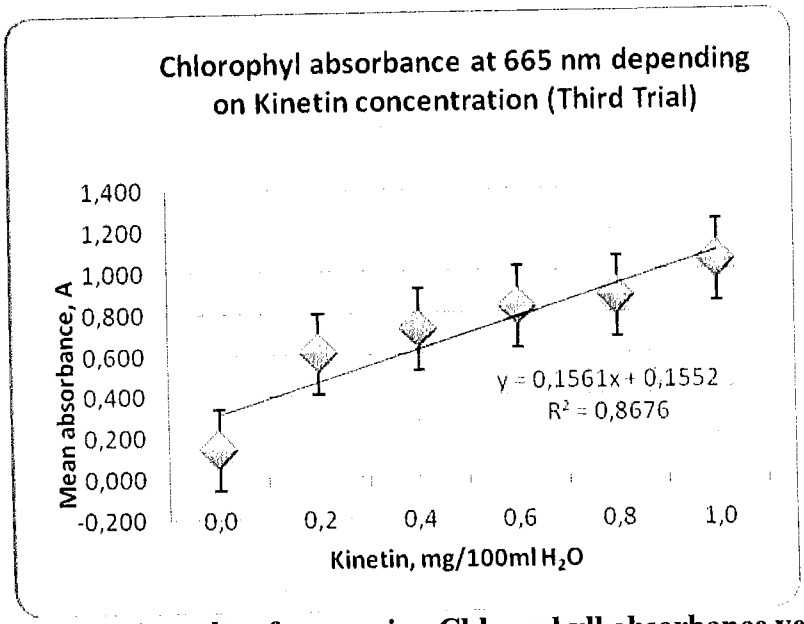
The Graph 2 represents a linear relationship between different Kinetin concentrations and the Mean absorbance (A) of the control probe and the extracts from the treated leaves of the second trial.

THIRD TRIAL

	ABSORBANCE OF THE SUSPENDED EXPLANTS AT 665nm					
	Control Probe, d H20	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
Mean ($\bar{X}$)	0,139	0,603	0,719	0,826	0,875	1,047
Standard deviation	0,067	0,026	0,058	0,196	0,075	0,188

Table 3: Barley leaf explants, treated with different concentrations of Kinetin (Third trial)

The results from the Table 3, give information about the mean value of the probes.



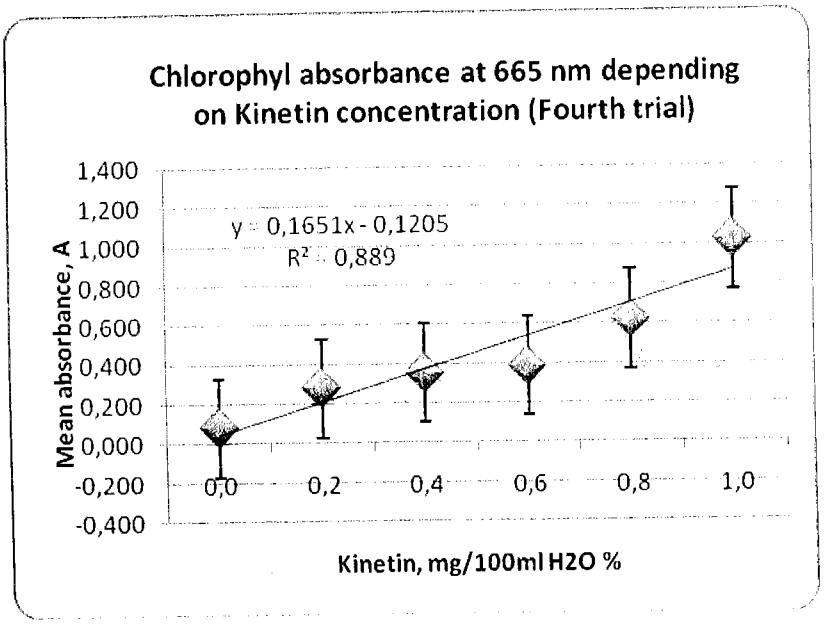
Graph 3. Experimental results of measuring Chlorophyll absorbance versus different concentrations of Kinetin (Third Trial)

The graphically represented data from the Third trial (Graph 3) show a linear relationship between Kinetin concentration and Chlorophyll absorbance.

FOURTH TRIAL

	ABSORBANCE OF THE SUSPENDED EXPLANTS AT 665nm					
	Control Probe, d H20	0,2 mg/100 ml KN	0,4 mg/100 ml KN	0,6 mg/100 ml KN	0,8 mg/100 ml KN	1,0 mg/100 ml KN
Mean ($\bar{X}$)	0,080	0,277	0,355	0,387	0,622	1,022
Standard deviation	0,043	0,035	0,029	0,125	0,158	0,252

Table 4: Barley leaf explants, treated with different concentrations of Kinetin (Fourth trial)



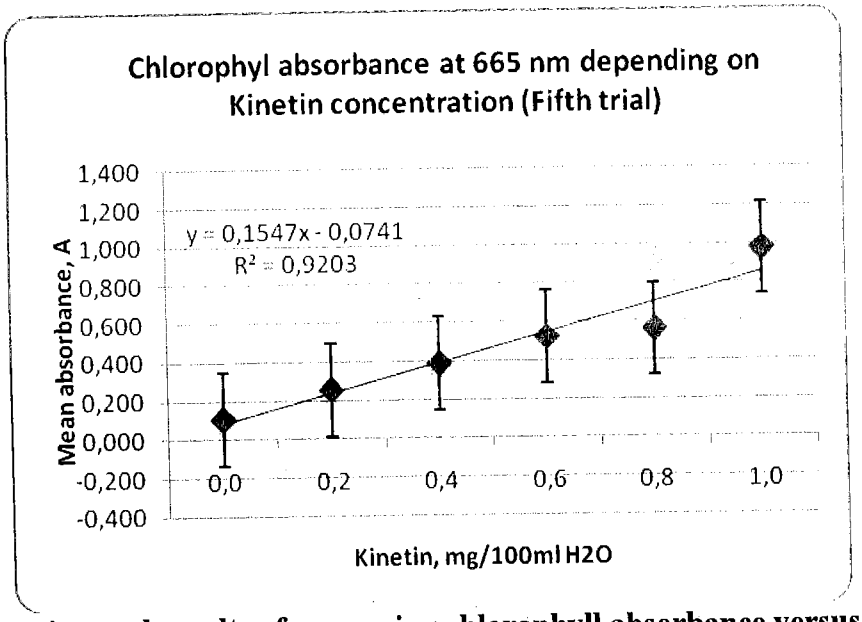
Graph 4. Experimental results of measuring Chlorophyll absorbance versus different concentrations of Kinetin (Fourth Trial)

The Graph 4 represents the investigated relationship (between the concentration of Kinetin and Chlorophyll absorbance) as linear one.

FIFTH TRIAL

	ABSORBANCE OF THE SUSPENDED EXPLANTS AT 665nm					
	Control Probe, d H20	0,2 mg/100 ml KN	0,4 mg/100 ml KN	0,6 mg/100 ml KN	0,8 mg/100 ml KN	1,0 mg/100 ml KN
Mean ($\bar{X}$)	0,105	0,253	0,387	0,522	0,559	1,029
Standard deviation	0,023	0,031	0,087	0,099	0,120	0,241

Table 5: Barley leaf explants, treated with different concentrations of Kinetin (Fifth trial)



Graph 5: Experimental results of measuring chlorophyll absorbance versus different concentrations of Kinetin (Fifth Trial)

Uncertainties:

During the experimental work several uncertainties could appear or already exist. They could lead to some anomalies in the results. That's why I've decided to calculate some of them.

➢ Calculating percentage uncertainty of cutting leaf explants

The formula for percentage uncertainty is written below:

$$\%\ uncertainty = \frac{uncertainty\ of\ the\ measuring\ instrument}{the\ volume\ of\ the\ substance\ measured}$$

During cutting the leaves an error could be made. So the percentage uncertainty made of this error is calculated below:
The uncertainty of the ruler is equal to ± 1mm
The length of the cut leaves was equal to 2cm = 20mm (it will occupy the place of the *volume of the substance measured in the denominator* of the formula above)
The percentage uncertainty made was calculated as follows:

$$\frac{1}{20} x100 = \frac{100}{20} = 5\%$$

This uncertainty will remind a constant because all the leaves were operated this way.

➢ Calculating percentage uncertainty of preparation Kinetin's solutions with different concentrations

The percentage uncertainty gained while making a Kinetin's solution with a definite concentration is calculated as a sum of the value of the percentage uncertainty of weighting specific quantity of Kinetin by using an electronic balance and the value of the volume of the dH_2O added to the solution by using a measuring flask.

Example for the first prepared solution with a concentration 0,2 mg/100ml Kinetin:

The solution is prepared as 1 mg (0,001g) Kinetin is dissolved in 500ml dH_2O.

1. The uncertainty of the electronic balance is equal to $\pm 0,0001$ (it is a constant).

   The percentage uncertainty of weighting 1 mg Kinetin by using an electronic balance will be calculated as follows:

$$\frac{\pm 0,0001}{0,001} \times 100 = 10\%$$

2. The percentage uncertainty made by the measuring flask will be calculated as follows:

The uncertainty of the measuring flask is equal to ±0,28ml
The volume of the distilled water added is equal to 500ml
Therefore the percentage uncertainty of the volume of dH_2O added to the solution will be:

$$\frac{\pm 0,25}{500} \times 100 = 0,05\%$$

This value will remain constant for all Kinetin solutions, because they were made in similar flasks.

3. The whole percentage uncertainty of the solution made will be gained when the value of percentage uncertainty of weighting specific quantity of Kinetin by using an electronic balance is summarized with the percentage uncertainty of the volume of dH_2O added to the solution by using a measuring flask:

0, 05% + 10, 00% =10, 05%

The same calculations were made for all the concentrations of Kinetin. The results are stated on the tables 12 to 16 in the columns named *Preparation of Kinetin solution.*

- Calculating percentage uncertainty of measuring the volume of Kinetin solution, used for treatment of the experimental probes

The other uncertainty that could be made when measuring the volume of Kinetin solution to moistened the experimental probes. This was done by measuring cylinder of 10 ml (±0, 1). The volume measured is equal to 10 ml.

Therefore the percentage uncertainty will be:

$$\frac{\pm 0,1}{10} \times 100 = 1\%$$

It will remain constant because for all probes, treated with different concentrations of Kinetin measuring cylinders with the same volume and uncertainties were used.

- Calculating percentage uncertainty of measuring Chlorophyll absorbance by using a spectrophotometer Selecta

The uncertainty made by the spectrophotometer for measuring the absorbance of the Chlorophyll is calculated as follows:

$$\%\ uncertainty = \frac{uncertainty\ of\ the\ measuring\ instrument}{chlorophyll\ absorbance\ measured}$$

Example of calculation the percentage uncertainty of Chlorophyll absorbance measured by a spectrophotometer:

The uncertainty of the spectrophotometer is equal to ±0,001

If the value of the Chlorophyll absorbance is equal to 0,152 (1st trial, 1st probe, Kinetin concentration = 0,2mg/100ml) the percentage uncertainty of the measurement is calculated as follows:

$$\%\ uncertanty = \frac{0,001}{0,152} x100 = 0,658\%$$

The calculations were done for all the trials separately.

For example, for the First trial all measurements of the Chlorophyll absorbance are shown in the table below.

	BARLEY LEAVES, TREATED WITH DIFFERENT CONCENTRATIONS OF KINETIN					
	Control Probe, d H20	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
First Trial	0,199	0,152	0,215	0,289	0,248	0,244
	0,198	0,138	0,242	0,263	0,271	0,228
	0,200	0,176	0,152	0,231	0,292	0,278
Mean ($\bar{X}$)	0,199	0,155	0,203	0,261	0,270	0,250

Table 6. Chlorophyll absorbance (A) of all probes and mean values.

The percentage uncertainties for the measurements from table 6 are given in the table 7.

	Percentage uncertainties of measuring Chlorophyll absorbance (First Trial)					
Probe Number	**Control Probe, d H20**	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
1	0,503	0,658	0,465	0,346	0,403	0,409
2	0,505	0,725	0,413	0,38	0,369	0,438
3	0,500	0,568	0,657	0,433	0,342	0,359
Mean	0,503	0,645	0,492	0,383	0,370	0,400

Table 7. Percentage uncertainties of measuring Chlorophyll absorbance by using a Spectrophotometer Selecta (processed data from the First trial)

In the table 7 are shown the uncertainties made by the spectrophotometer while measuring the absorbance of the leaf extracts at 665 nm. As it could be clearly seen from the Graph 1, all the results are in the boundaries of the error bars and there are no anomalies. This statement is well proved while looking at the low values of the uncertainties showed in table 7. The results also show a high precision of work.

For the next trials the mean values were only taken into consideration. They are representative to assess the uncertainties made by the spectrophotometer, and to lead to clear conclusions about the precision of the work.

In the tables 8, 9, 10 and 11 the uncertainties of measuring Chlorophyll absorbance of the second, third, fourth and fifth trial can be seen.

	Percentage uncertainties of measuring Chlorophyll absorbance (Second Trial)					
Probe Number	**Control Probe, d H20**	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
Mean value of Chlorophyll absorbance	0,259	0,566	0,685	0,859	0,977	1,095
% Uncertainty	0,386	0,177	0,146	0,116	0,0913	0,0899

Table 8. Percentage uncertainties of measuring Chlorophyll absorbance by using a Spectrophotometer (processed data from the Second trial)

	Percentage uncertainties of measuring Chlorophyll absorbance (Third Trial)					
Probe Number	**Control Probe, d H20**	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
Mean value of Chlorophyll absorbance	0,139	0,603	0,719	0,826	0,875	1,047
% Uncertainty	0,719	0,166	0,139	0,121	0,114	0,0955

Table 9. Percentage uncertainties of measuring Chlorophyll absorbance by using a Spectrophotometer (processed data from the Third trial)

	Percentage uncertainties of measuring Chlorophyll absorbance (Fourth Trial)					
Probe Number	**Control Probe, d H20**	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
Mean value of Chlorophyll absorbance	0,139	0,603	0,719	0,826	0,875	1,047
% Uncertainty	1,250	0,361	0,281	0,258	0,161	0,0978

Table 10. Percentage uncertainties of measuring Chlorophyll absorbance by using a Spectrophotometer (processed data from the Fourth trial)

	Percentage uncertainty of measuring Chlorophyll absorbance (Fifth Trial)					
Probe Number	**Control Probe, d H20**	**0,2 mg/100 ml KN**	**0,4 mg/100 ml KN**	**0,6 mg/100 ml KN**	**0,8 mg/100 ml KN**	**1,0 mg/100 ml KN**
Mean value of Chlorophyll absorbance	0,105	0,253	0,387	0,522	0,559	1,029
% Uncertanty	0,952	0,395	0,258	0,192	0,178	0,102

Table 11. Percentage uncertainties of measuring Chlorophyll absorbance by using a Spectrophotometer (processed data from the Fifth trial)

- Calculating summary of percentage uncertainties

%Uncertanty of the solution- 1st trial					
Concentration of Kinetin	**Uncertainty at the preparation of the solution**	**Uncertanty of the test tube used**	**Uncertanty of the spectrophotometer**	**Uncertanty of the cut leaves**	**Total uncertanty**
0,20%	10,050	1,000	0,645	5,000	16,695
0,40%	5,050	1,000	0,492	5,000	11,542
0,60%	3,350	1,000	0,383	5,000	9,733
0,80%	2,500	1,000	0,37	5,000	8,870
1,00%	2,050	1,000	0,400	5,000	8,450

Table 12. Calculated total percentage uncertainties for the First trial.

%Uncertanty of the solution – 2nd trial					
Concentration of Kinetin	Uncertainty at the preparation of the solution	Uncertanty of the test tube used	Uncertanty of the spectrophotometer	Uncertanty of the cut leaves	Total uncertanty
0,20%	10,050	1,000	0,177	5,000	16,227
0,40%	5,050	1,000	0,146	5,000	11,196
0,60%	3,350	1,000	0,116	5,000	9,466
0,80%	2,500	1,000	0,091	5,000	8,591
1,00%	2,050	1,000	0,089	5,000	8,139

Table 13: Calculated total percentage uncertainties for the Second trial.

%Uncertanty of the solution – 3rd trial					
Concentration of Kinetin	Uncertainty at the preparation of the solution	Uncertanty of the test tube used	Uncertanty of the spectrophotometer	Uncertanty of the cut leaves	Total uncertanty
0,20%	10,050	1,000	0,166	5,000	16,216
0,40%	5,050	1,000	0,139	5,000	11,189
0,60%	3,350	1,000	0,121	5,000	9,471
0,80%	2,500	1,000	0,114	5,000	8,614
1,00%	2,050	1,000	0,096	5,000	8,146

Table 14. Calculated total percentage uncertainties for the Third trial.

%Uncertanty of the solution- 4th trial					
Concentration of Kinetin	Uncertainty at the preparation of the solution	Uncertanty of the test tube used	Uncertanty of the spectrophotometer	Uncertanty of the cut leaves	Total uncertanty
0,20%	10,050	1,000	0,361	5,000	16,411
0,40%	5,050	1,000	0,281	5,000	11,331
0,60%	3,350	1,000	0,258	5,000	9,608
0,80%	2,500	1,000	0,161	5,000	8,661
1,00%	2,050	1,000	0,098	5,000	8,148

Table 15. Calculated total percentage uncertainties for the Fourth trial.

%Uncertanty of the solution- 5th trial					
Concentration of Kinetin	Uncertainty at the preparation of the solution	Uncertanty of the test tube used	Uncertanty of the spectrophotometer	Uncertanty of the cut leaves	Total uncertanty
0,20%	10,050	1,000	0,359	5,000	16,409
0,40%	5,050	1,000	0,258	5,000	11,308
0,60%	3,350	1,000	0,192	5,000	9,542
0,80%	2,500	1,000	0,178	5,000	8,678
1,00%	2,050	1,000	0,102	5,000	8,152

Table 16. Calculated total percentage uncertainties for the Fifth trial.

The table below shows the total values of the uncertainties made.

Total uncertanties for all the trials made				
Total - 1st Trial	Total - 2nd Trial	Total - 3rd Trial	Total - 4th Trial	Total - 5th Trial
16,695	16,227	16,216	16,411	16,409
11,542	11,196	11,189	11,331	11,308
9,733	9,466	9,471	9,608	9,542
8,870	8,591	8,614	8,661	8,678
8,450	8,139	8,146	8,148	8,152

Table 17. Total uncertainties for all trials

The results were calculated by Microsoft Office Excel.

Analysis and Evaluation

Analysis of data and graphs:

1st Trial:

The data analysis from Table 1 shows a stable tendency of increasing absorbance at 665 nm when the probes are treated with increasing concentrations of Kinetin solutions. One exception is the final concentration of Kinetin (1,0mg/100ml) at which the mean absorbance of 0,250 is slightly lower than those values at the other concentrations. The absorbance is also higher in the control probe in comparison with probes, treated with 0, 2mg/100ml Kinetin.

The Graph 1 represents the relationship between concentration of Kinetin solutions and the absorbance of the control probe and the treated probes at 665nm. The graph expresses a linear dependence. The coefficient R=62% give information that the experimental results coincide approximately 62% with the theoretically expected ones. This R-value isn't very high and probably may be explained with low precision of experimental work for the first time. Nevertheless, the experimental results follow the trend line and all of the points on the graph lie in the boundaries of the error bars, which mean that there are no anomalous points. In order to increase the reliability of the experiment supporting the alternative hypotheses more repeats were don

2nd Trial:

According to the results from table 2, the mean values of the Chlorophyll absorbance, increase with the increase of the Kinetin concentrations in the experimental probes. The absorbance is also higher in each of them in comparison with the control probe. These results unambiguously prove that when treated with the growth regulator Kinetin, the leaf explants synthesize more Chlorophyll, which means that they activate their metabolic processes and maintain their vitality. This statement rejects the zero hypothesis (H_0) according to which the Kinetin does not affect the biosynthesis of Chlorophyll and the aging of cut leaves. The values of *Standard deviation* vary between 0,129 and 0,254 in the experimental probes versus 0,135 in the control probe. Those values are small and indicate for a high precision of the experimentally collected data as well as signify that the data show a normal distribution around the mean value.

The Graph 2 represents a linear relationship between different Kinetin concentrations and the absorbance of the extracts of the control probe and the experimental probes from the second trial of the experiment. The coefficient R is equal to 96%, which means that the approximation of the experimental results to the theoretically expected ones is very high. This is also and evidence for the reliability of the experiment and truthfulness of the alternative hypotheses.

The values of Standard deviations (SD) are used the error bars to be created. The trend line is within all the error bars, which signifies that the data is reliable.

The probes treated with 0,8 and 1,0mg/100ml Kinetin have values of SD higher than the previous three, which means that the fluctuation of the dependent variable (Chlorophyll absorbance) around the mean value in those probes is higher.

The control probe has the lowest Chlorophyll absorbance, when compared with the experimental probes, treated with Kinetin. Nevertheless, the SD values for all probes are low enough to consider that the data are normally distributed around the means.

3rd Trial

The analysis of the data from Table 3 gives information that with the increase of the Kinetin concentration in the experimental probes, the mean value of the absorbance also increases..

When comparing the mean absorbance of the experimental probes with this of the control probe, it is obvious that the control probe has a very low value of the mean absorbance. This can be explained with the lack of Kinetin, which is a factor that stimulates the biosynthesis of Chlorophyll.

The lowest value of chlorophyll absorbance is in the probe, treated with 0,2mg/100 ml Kinetin and the highest one-in the probe, treated with 1,0mg/100 ml Kinetin. This shows that the value of the Chlorophyll absorbance is higher, when the leaf explants were treated with higher Kinetin concentration. These experimental results clearly support the alternative hypothesis (H_A), according to which the Chlorophyll level will increase while increasing the Kinetin concentration. The values of SD are not high (small than 1 unit), which indicates that there is a normal distribution of the experimental data around the mean values for each experimental probe.

The graphically represented data from the Third trial (Graph 3) show a linear relationship between Kinetin concentration and Chlorophyll absorbance. The value of the coefficient R, which is equal to approximately 87%, indicates for a high coincidence of the experimental data with the theoretically expected ones. The point on the graph, corresponding to the control probe is positioned down to the points, representing the experimental probes, but it is still in the boundaries of the error bars and thus it must not be considered as an anomalous one.

4th Trial:

The experimental data from the Fourth trial show again a stable tendency of increased Mean Chlorophyll absorbance with the increase of the Kinetin concentration. The Standard deviation is bigger in the probes, treated with higher concentrations of Kinetin (0,6; 0,8 and 1,0mg/100ml). This is due to high fluctuations of the experimental results around the mean values. It might also be an indicator that different leaf explants respond differently to the treatment.

Anyway the Graph 4 represents the investigated relationship as linear one. The coefficient R is equal to approximately 89%, which is a high level of coincidence with the trend line.

5th Trial:

The results from the Table 5 show a clear and stable tendency between the Kinetin concentration and the Chlorophyll absorbance. There is a notable leap of the Chlorophyll absorbance at the highest Kinetin concentration (1,0mg/100ml), which leads to lower standard deviation. The data from the Table 5 show that the increased concentration of Kinetin leads to increased absorbance of the suspended explants. An interesting fact is that for each of the probes treated with different concentrations of Kinetin (0,6; 0,8; 1,0mg/100ml) the Mean Chlorophyll absorbance significantly differs from the control probe. The high coincidence (R = 92%) of the experimental data with the trend line signifies for the precision of work and also shows the reliability of the trial and the alternative hypotheses (H_A).

The table below shows the mean values and standard deviations of all trials. The great number of trials aims at showing bigger reliability.

		Standard deviations and mean values of the Chlorophyll absorbance					
		Control Probe, d H20	0,2 mg/100 ml KN	0,4 mg/100 ml KN	0,6 mg/100 ml KN	0,8 mg/100 ml KN	1,0 mg/100 ml KN
First Trial	Mean ($\bar{X}$)	0,199	0,155	0,203	0,261	0,270	0,250
	Standard Deviation		0,027	0,064	0,023	0,015	0,035
Second Trial	Mean ($\bar{X}$)	0,259	0,566	0,685	0,859	1,095	1,112
	Standard Deviation	0,135	0,129	0,149	0,121	0,254	0,205
Third Trial	Mean ($\bar{X}$)	0,139	0,603	0,719	0,826	0,875	1,047
	Standard Deviation	0,067	0,026	0,058	0,196	0,075	0,188
Forth Trial	Mean ($\bar{X}$)	0,080	0,277	0,355	0,387	0,622	0,102
	Standard Deviation	0,043	0,035	0,029	0,125	0,158	0,252
Fifth Trial	Mean ($\bar{X}$)	0,105	0,253	0,387	0,522	0,559	0,977
	Standard Deviation	0,023	0,031	0,087	0,099	0,120	0,241

Table 18. Mean values and standard deviations for all the trials

The analysis of the experimental results of all trials, show a stable relationship between the Chlorophyll absorbance at 665 nm and the Kinetin concentration. This correlation shows that the biggest Kinetin concentration delays the plant's aging and fading. Also the high values of the coefficient R, show the coincidence between the experimental data and the theoretically expected ones, indicate for a high level of coincidence between the experimental results and the trend line in almost all of the trials. This is a reason the experiment to be considered as reliable (the data from tables and graphs 1 to 5 clearly express this tendency).

The standard deviation (SD) of the results gained is very low in all of the trials and all the probes, which shows a high precision of the work through doing the experiment.

T-test – analysis

In order to prove the Alternative hypothesis (H_A), according to which the growth regulator Kinetin prevents the aging of cut leaves, the experimental results were tested by using the statistical **t-test**.

The formula for the *t-test* is:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{(n-1)\ s_x^2 + (m-1)s_y^2}{n+m-2}\left(\frac{1}{n} + \frac{1}{m}\right)}}$$

The table below shows the data obtained by t-test:

	Control Probe, d H_2O	0,2 mg/100 ml Kinetin	0,4 mg/100 ml Kinetin	0,6 mg/100 ml Kinetin	0,8 mg/100 ml Kinetin	1,0 mg/100 ml Kinetin
	0,199	0,152	0,215	0,289	0,248	0,244
	0,198	0,138	0,242	0,263	0,271	0,228
	0.200	0,176	0,152	0,231	0,292	0,278
	0,210	0,633	0,585	0,730	1,367	0,921
	0,262	0,418	0,614	0,875	0,863	1,086
	0,306	0,648	0,857	0,971	1,056	1,328
	0,206	0,588	0,777	1,051	0,788	0,830
	0,073	0,633	0,662	0,736	0,924	1,163
	0,137	0,588	0,717	0,690	0,912	1,147
	0,030	0,267	0,373	0,252	0,600	0,842
	0,110	0,315	0,370	0,409	0,477	1,310
	0,099	0,248	0,322	0,500	0,790	0,914
	0,096	0,260	0,400	0,411	0,654	1,252
	0,088	0,220	0,295	0,556	0,425	0,806
	0,131	0,280	0,467	0,599	0,599	0,872
T-test value	control	0,000676	0,000505	0,000216	0,000104	0,000017
p-value	control	p<0,05	p<0,05	p<0,05	p<0,05	p<0,05

Table 19. T-test results

All the concentrations of Kinetin were separately compared with the control probe. The t-test shows whether the zero hypotheses (H_0) are true or not. If the p-value is lower than 0, 05, this indicates that the H_0 is rejected and the alternative one (H_A) should be taken into consideration. The p-value lower than 0, 05 also means that the difference between the experimental probe and the control probe is statistically significant.

As the calculations for the p-values are seen from the Table 19 for all trials this value is lower than 0, 05, which means that there is a significant difference between the control probe and the probes treated with different concentrations of Kinetin. Therefore the zero hypotheses (H_0), according to which the growth regulator Kinetin doesn't affect leaves' aging, should be rejected and the alternative ones (H_A) should be accepted.

An interesting fact is that with the increase of the Kinetin concentration the statistical significance of the differences between the experimental probes and the control probe increases. This supports one of the Alternative hypotheses (H_A), which state that the increased Kinetin concentrations decrease the aging of the cut leaves.

Analysis of the uncertainties:

During the experimental work several uncertainties could arise. Examples are given in the section *Uncertainties*. The apparatus used has an acceptable accuracy, but for the percentage uncertainty of weighting 2 mg Kinetin by using an electronic balance, the values calculated were higher than it was expected. This can be seen in the subsection "Calculating percentage uncertainty of preparation Kinetin's solutions with different concentrations-*Example for the first prepared solution with a concentration 0, 2 mg/100ml Kinetin"*. Despite the fact that the accuracy of the electronic balance is equal to ±0, 0001, the percentage uncertainties of the weighting Kinetin by an electronic balance, are in the boundaries of 2, 05% and 10, 05%. These are the highest percentage uncertainties for the whole experiment. A reason for that could be the low mass of Kinetin measured by the electronic balance (0,2mg KN/100ml H_2O; 0,4mg KN/100ml H_2O; 0,6mg KN/100ml H_2O; 0,8mg KN/100ml H_2O; 1,0mg KN/100ml H_2O). Anyway this is not an obstacle for the exactness of the results gained, because the other uncertainties gained are lower.

The uncertainty of the spectrophotometer could be taken for example. They are very low. This could be well proved while looking at the tables 7-11, which show the percentage uncertainty of measuring Chlorophyll absorbance. The uncertainties vary in the boundaries of 1, 250 (4^{th} trial, the uncertainty made by the spectrophotometer for the control probe) and 0,089 (2^{nd} trial, the probe of 1mg Kinetin/ 100 ml H_2O).

The values of percentage uncertainties become lower at higher concentrations of Kinetin. The reason for that is that the highest value of Chlorophyll absorbance was gained for the probes treated with 1,0 mg Kinetin/ 100ml H_2O. This statement also answers the research question- *How does the growth regulator Kinetin, affect plant's aging?*- in such a way that with the increase of the Kinetin concentration in the probes, there is more Chlorophyll and therefore- the leaves' life was protracted.

In the tables 12 to 16 all the uncertainties for all the trials separately are summarized. Table 17 shows the total values of uncertainties made in all the trials. It could be clearly seen that with the increase of the concentration of Kinetin in the probes, the uncertainties' values become lower and therefore. This signifies for more accurate and more reliable experimental results.

Improvements

As it was summarized in the section *Analysis and Evaluation,* the method worked out and the trials are considered successful.

The results gained of the experiment presented in this Extended Essay and the low values of the uncertainties show high precision of work while doing the experiment. But anyway it could be well improved while doing it with higher concentrations of Kinetin. This way the uncertainties of measurements by the electronic balance will be lower and therefore the results- more accurate.

It will be a good decision if the results were measured at different wave length (by using the spectrophotometer). The measurement of the absorbance of Chlorophyll A and Chlorophyll B at two wavelengths will utilize the calculation of concentration of the Chlorophyll in the investigated probes.

On the other hand, Barley leaves were only used in the experiment, so clear conclusions could be made only for the impact of Kinetin on the barley leaves and only for the concentrations of Kinetin, which were applied. It will be a good idea if other plants are taken for the experiment as well as other concentrations are examined. This way the experimental results could be compared and thus more reliable the confirmation of the Alternative hypotheses (H_A) will be.

Additionally, the experiment could be done with a great variety of Kinetin concentrations. This will give extra information, which could be useful for improving the experiment. This way the experiment will be complete and highly reliable.

Conclusion

As a conclusion it can be said that the results gained and observed answer to my with my research questions. It clearly shows that the highest concentration delays the ~~loose~~ of chlorophyll and therefore it delays the ageing. This assertion itself proves that the zero hypotheses- H_0, which discard any effect of the Kinetin on the leaves' aging, will be rejected; so the alternative hypotheses- H_A, will be accepted. My prediction that the growth regulator Kinetin will show its properties as a growth regulator was well proved looking at the results gained. They show a dependency between the concentration of Kinetin and the Chlorophyll absorbance. The Chlorophyll level measured by the spectrophotometer, increases with the increasing of the Kinetin concentration, which therefore proves my second alternative hypothesis that the higher concentration of Kinetin, slows down the aging of the cut leaves. Also it proves my expectation that the Chlorophyll level will increase with the increasing of the Kinetin concentration. This can be clearly seen from Graps 1-5.

The results shown in this Extended essay may help for the better understanding and improvement of the knowledge about the nature, the properties of the Kinetin, and especially- the effect of the growth regulator on the leaves' aging.

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Appendix

Data results for all trials:

	BARLEY LEAVES, TREATED WITH DIFFERENT CONCENTRATIONS OF KINETIN-FIRST TRIAL					
	Control Probe, d H20	0,2mg Kinetin/100ml H_2O	0,4mg Kinetin/100ml H_2O	0,6mg Kinetin/100ml H_2O	0,8mg Kinetin/100ml H_2O	1,0 mg Kinetin/100ml H_2O
First Trial	0,199	0,152	0,215	0,289	0,248	0,244
	0.198	0,138	0,242	0,263	0,271	0,228
	0.200	0,176	0,152	0,231	0,292	0,278
Mean absorbance (X)	0,199	0,155	0,203	0,261	0,270	0,250
Standard deviation		0,027	0,064	0,023	0,015	0,035
Second Trial	0,210	0,633	0,585	0,730	1,367	0,921
	0,262	0,418	0,614	0,875	0,863	1,086
	0,306	0,648	0,857	0,971	1,056	1,328
Mean absorbance (X)	0,259	0,566	0,685	0,859	1,095	1,112
Standard deviation	0,135	0,129	0,149	0,121	0,254	0,205
Third Trial	0,206	0,588	0,777	1,051	0,788	0,830
	0,073	0,633	0,662	0,736	0,924	1,163
	0,137	0,588	0,717	0,690	0,912	1,147
Mean absorbance (X)	0,139	0,603	0,719	0,826	0,875	1,047
Standard deviation	0,067	0,026	0,058	0,196	0,075	0,188
Fourth Trial	0,030	0,267	0,373	0,252	0,600	0,842
	0,110	0,315	0,370	0,409	0,477	1,310
	0,099	0,248	0,322	0,500	0,790	0,914
Mean absorbance (X)	0,080	0,277	0,355	0,387	0,622	1,022
Standard deviation	0,043	0,035	0,029	0,125	0,158	0,252
Fifth Trial	0,096	0,260	0,400	0,411	0,654	1,252
	0,088	0,220	0,295	0,556	0,425	0,806
	0,131	0,280	0,467	0,599	0,599	0,872
Mean absorbance (X)	0,105	0,253	0,387	0,522	0,559	0,977
Standard deviation	0,023	0,031	0,087	0,099	0,120	0,241

Table 1: All the trials and all the results

T-test

Control Probe, H_2O	0,20 % Kinetin
0,199	0,152
0.198	0,138
0.200	0,176
0,210	0,633
0,262	0,418
0,306	0,648
0,206	0,588
0,073	0,633
0,137	0,588
0,030	0,267
0,110	0,315
0,099	0,248
0,096	0,260
0,088	0,220
0,131	0,280

Table 2: Results obtained by t-test for 0,20% Kinetin

The t-test in this case showed the results:

t-test value= $6{,}76 \times 10^{-4}$
Therefore the p-value is lower than 0,5
p-value<0,5

The table below shows the results obtained by t-test for 0,40% Kinetin:

Control Probe, H_2O	0,40% Kinetin
0,199	0,215
0.198	0,242
0.200	0,152
0,210	0,585
0,262	0,614
0,306	0,857
0,206	0,777
0,073	0,662
0,137	0,717
0,030	0,373
0,110	0,370

0,099	0,322
0,096	0,400
0,088	0,295
0,131	0,467

Table 3: Results obtained by t-test for 0,40% Kinetin

t-test value: $5{,}047 \times 10^{-5}$
p-value <0,5

The table below shows the results obtained by t-test for 0,60% Kinetin:

Control Probe, H_2O	**0,60% Kinetin**
0,199	0,289
0.198	0,263
0.200	0,231
0,210	0,730
0,262	0,875
0,306	0,971
0,206	1,051
0,073	0,736
0,137	0,690
0,030	0,252
0,110	0,409
0,099	0,500
0,096	0,411
0,088	0,556
0,131	0,599

Table 4: Results obtained by t-test for 0,60% Kinetin

t-test value: $2{,}16133 \times 10^{-5}$
⇨ p-value < 0,5

The table below shows the results obtained for 80% Kinetin:

Control Probe, H_2O	**0,80% KN**
0,199	0,248
0.198	0,271
0.200	0,292
0,210	1,367
0,262	0,863
0,306	1,056
0,206	0,788
0,073	0,924
0,137	0,912
0,030	0,600
0,110	0,477

0,099	0,790
0,096	0,654
0,088	0,425
0,131	0,599

Table 5: Results obtained by t-test for 0,80% Kinetin.

t-test value: $1{,}04146 \times 10^{-5}$

⇨ p-value<0,5

The table below shows the results obtained by t-test for a concentration of Kinetin=1%.

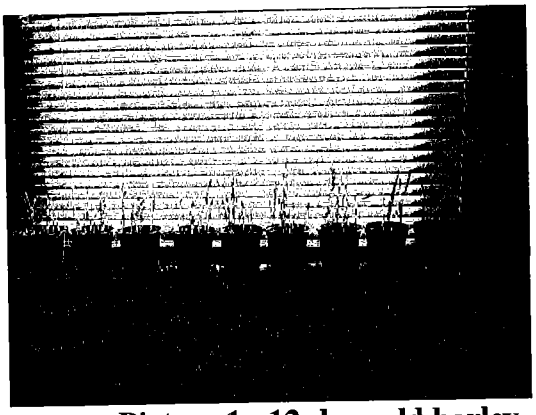
Control Probe, H_2O	**1,00% KN**
0,199	0,244
0.198	0,228
0.200	0,278
0,210	0,921
0,262	1,086
0,306	1,328
0,206	0,830
0,073	1,163
0,137	1,147
0,030	0,842
0,110	1,310
0,099	0,914
0,096	1,252
0,088	0,806
0,131	0,872

Table 6: Results for control probe- H_2O and 1% Kinetin

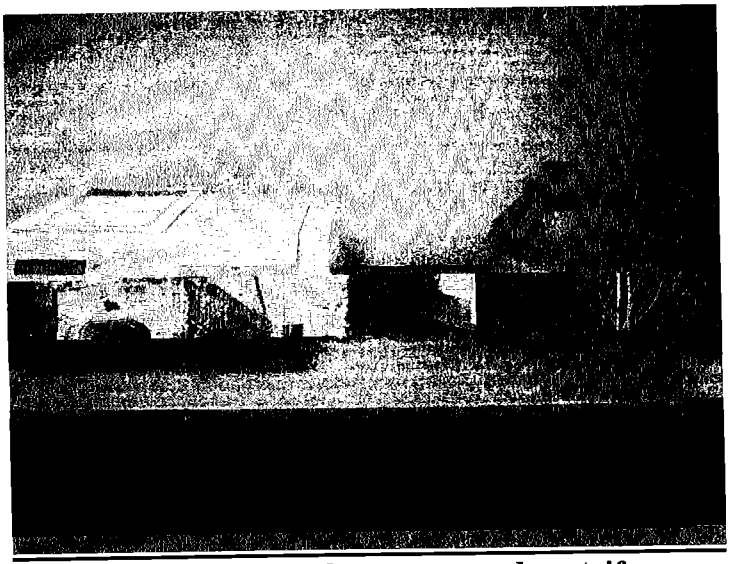
t-test value: $1{,}675 \times 10^{-5}$

⇨ p-value<0,5

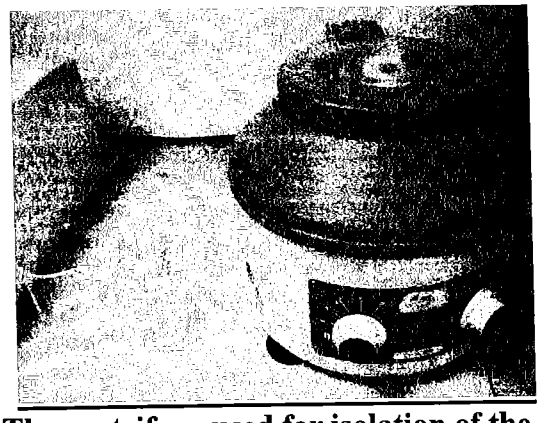
Pictires



Picture 1 : 12-days old barley



Picture 2: Spectrophotometer and centrifuge.



Picture 3: The centrifuge used for isolation of the Chlorophyl