TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Effect of aspartame on the growth and health of *Portulaca Oleracea*

Biology Extended Essay

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ABSTRACT

This experiment is based on the growth of the plant *Portulaca Oleracea* under the effect of different number of aspartame tablets added for watering. Aspartame is an artificial sweetening agent which gives the same taste of sugar both in a cheaper and effective way. To test the toxic effects of aspartame I have choosen the plant *P. Oleracea* as it grows easily in a short period of time and it is a eukaryotic organism like human beings. My research question is: "**Does adding aspartame solution in different concentrations to a pot of *P. Oleracea* affects the rate of growth and health of the plant which are indicated by measuring the change in length and counting number of leaves respectively, within 20 days"**

In the experiment, I planted the *P. Oleracea* plants into five identical pots and watered each of them with different amounts of dissolved aspartame. I prepared the solution for watering under standard conditions and I also prepared 0.1M NaOH solution and added it to the main solution to stabilize the pH level to 7.

In order to justify my results and see if they are valid or not, I used Anova Single Factor for analization. The P-value obtained from the lengths of the plants was 5.0469E-19 and 1.24E-14 for number of leaves which supports my hypothesis by being less than 0.05.

This experiment showed that aspartame effects the growth and number of leaves of the plant in a negative way. So natural sweeteners should be used instead of the artificial sweetener aspartame, which gives harm to living eukaryotic cells.

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I. INTRODUCTION

Artificial sweeteners are used by many people especially people with diabetes to decrease the amount of carbohydrate intake and by this way to decrease the blood glucose level. One of the most common sweetener is “aspartame”. Aspartame is a methyl ester of the aspartic acid/phenylalanine dipeptide. It is defined as an artificial, non-saccharide sweetener used as sugar substitute in some foods and beverages.

Aspartame was discovered in 1965 by James M. Schlatter, a chemist working for G.D. Searle & Company. He had synthesized aspartame as an intermediate step in generating a tetrapeptide of the hormone gastrin, for use in assessing an anti-ulcer drug candidate. When he licked his finger he accidentally discovered its sweet taste.

Aspartame lets diabetes people have greater variety and flexibility in budgeting their total carbohydrate intake and allows them to satisfy their taste for sweets without affecting blood sugar, which helps for a healthful meal plan. In addition, consuming products with aspartame can result in fewer calories, which helps not only people with diabetes but also other people using aspartame to manage a healthful diet.

It appears that it is the mostly used sweetener by diabetic patients because of its low cost and highly sweet taste. That much of sweetness grabs attention of people in cake and desert business so that it has highly usage in those.

In 1980, the FDA convened a Public Board of Inquiry (PBOI) consisting of independent advisors charged with examining the relationship between aspartame and brain cancer. The PBOI concluded aspartame does not cause brain damage, but it recommended against approving aspartame at that time, citing unanswered questions about cancer in laboratory rats.[1]

This matter is found in thousands of different food products and beverages because of being about 200 times sweeter than normal sugar, so much less of it can be used to give the same taste of sweetness. By this way, it also lowers the calories in the food.[2]

I have widened my research on that topic. The most interesting part was aspartame never really defined as a cause of cancer. It seems that although few cancerous signs observed, it could never been linked to the usage of the sweetener because of the lack of evidences. Many lab studies looked for effects in animals fed with aspartame, often in high doses each day over their lifetimes. None of these studies have found health issues that are consistently linked with aspartame.

There are over 92 different side effects related with aspartame consumption. According to Lendon Smith, M.D. a majority is suffering from these, yet has no idea why drugs, supplements and herbs don't relieve their symptoms.

Side effects of aspartame include:

- Blindness
- Ringing or buzzing sounds in ear
- Headaches, migraines (some severe)
- Severe depression
- Itching without a rash on skin[3]

[1] http://en.wikipedia.org/wiki/Aspartame
[2] http://www.cancer.org/cancer/cancercauses/othercarcinogens/athome/aspartame
[3] http://www.sweetpoison.com/aspartame-side-effects.html

While there were several studies on the effect of aspartame on living cells, I decided to test it by myself using a plant called *P. Oleracea*. The saplings were easy to grow and have similar cellular characteristics with human cells as it is a eukaryotic organism. Common name of it is Purslane in the US; other names that can be known in different places are, like Pigweed, garden purslane etc.[4]

Because of its no-fuss-needed nature this plant is widespread all over the globe. It mostly grows in, vegetable gardens, agricultural lands, lawns, unused pathways having basically warm weathered zones.[5] This plant has several qualities such as being used in the preparation of medicines and food items. With its high nutrient value these plants have high capacity of abrogating certain health problems in both natural and effective way.

I have planned to observe the toxic effects of different concentrations of aspartme on some phenotypical characteristics of *P. Oleracea* such as length and number of leaves in a defined period of time. Thereby, this paper will focus on: **Does adding aspartame solution in different concentrations (0, 3, 6, 10, 15 aspartame tablets in 120 ml of water) to *P. Oleracea* effects the rate of growth of the plant and total number of leaves which are indicated by measuring the change in length and counting number of leaves for twenty days under a specific environmental conditions.**

I will also discuss how the experiment held, prepared and performed with examination results obtained in order to evaluate the validity.

[4] http://en.wikipedia.org/wiki/Aspartame
[5] http://www.onlyfoods.net/portulaca-oleracea.html

II. HYPOTHESIS

Cancer may occur due to several toxic agents that we are faced in our daily lives. These toxic agents can harm our cells and tissues. Effected cells can lose their normal characteristics, like normal cell growth.

There is evidence that aspartame has some side effects which can be harmful to a living cell. As I mentioned in introduction part, I will use *P. Oleracea* to observe whether aspartame causes toxic effects or not, by observing the growth of the plant and number of leaves that plants have in twenty days.

Therefore it can be hypothesized that as the concentration of aspartame increases, the rate of growth and number of leaves of plant will decrease. It is predicted that as the dissolved aspartame amount in solution increases, it will decrease both the rate of growth and number of leaves of the *P. Oleracea* because of the toxic effects of the aspartame on living cells.

III. METHOD DEVELOPMENT AND PLANNING

My mother uses an artificial sweetening agent while she drinks tea. She is using Sanpa© brand sweetening tablets. I checked the ingredient list from its label and the active ingredient of it was "aspartame". I have always wondered that how can an artificial agent give exactly the same taste with the natural sugar and does this agent cause any harm or toxic effect to living organisms. So I have decided to make an investigation to see the effects of aspartame.

In order to support or reject the hypothesis "As the concentration of aspartame increases, the rate of growth of plant and number of leaves that plant have will decrease" I have chosen the plant *P. Oleracea* and the sweetener agent aspartame. Five different concentrations of aspartame solutions which include 3, 6, 10, 15 aspartame tablets in 120 ml water and one control sample without any tablets were used to water the plants. I decided to make a control sample in order to make sure that any toxic side effects observed on plant is caused by aspartame tablets added to water. So that aspartame will be the only variable that affects the plant cells.

First reason why I have chosen Sanpa© aspartame as the sweetener to use in my experiment is that it is the brand that my mother always uses in her drinks. Moreover, it can be obtained with low prices from the market. Aspartame has the ability to intensify and extend fruit flavors, like orange and cherry, in foods and beverages. For instance, sweet taste of chewing gum comes from aspartame and also its flavor lasts longer than sugar-sweetened gum.[6]

[6] http://www.aspartame.org/aspartame_facts_brochure.html

Obviously there is an actual need to observe the toxic effects of the aspartame. It could not be observed in humans as it won't be ethical and couldn't be on any vertebrate for the same reason. So I have chosen *P. Oleracea* plant which is a eukaryotic organism to test the toxic effects of aspartame. Being a eukaryotic organism this plant shows similar patterns with animal cells. Even chlorophyll molecule shows a slight difference with hemoglobin found in human cells. That is why, I didn't choose a bacterium which is prokaryotic.

I didn't have any laboratory conditions. So I couldn't work with fungi or yeast too, as they require a well-equipped microbiology lab. Other advantages of using *P. Oleracea* are;

- Fast growth period
- Easy to plant buds
- Easy to take care
- No special nutrient need to cultivate

I will observe the change in length of plant and number leaves on the plant by watering the plants with water samples that include different numbers of aspartame tablets. The lifetime of the plants will also be noted down in order to further investigate the effect of aspartame on the life time of the plant. Throughout this experiment the biggest problem causing variable was the pH change in the solution which I was giving to the plant. The dissolved aspartame tablets in water make it more acidic with each addition so in order to adjust any change in pH, I added drop wise 0.1M NaOH (Merck©) solution to each aspartame solution and adjusted the final pH of the solution to 7. Before each watering, pH of the solution is checked and noted down by using pH meter.

Furthermore; to stabilize the conditions like temperature and pressure, I put all the samples in the same room where all of them will take the same light intensity during the day. I used a thermometer for each of the plants in order to make sure that they are under same conditions. This standard room condition also helped me while keeping CO_2 availability for each plant constant.

All materials such as; water (volume and type), plant (initial length, mass, type, leaves), soil (volume and type), pots (volume and type) are taken identical in order to avert any effects that can result from using different materials on different samples. The humidity of the soil is kept constant by taking all soil needed from the same package and keeping them under the same room conditions.

Before starting the experiment I have to decide the amount of aspartame that I am going to test and find out how much water does a *P. Oleracea* plant requires. As I mentioned my mother uses these sweeteners in her daily routine. So I will set the amount of aspartame addition based on her usage. She uses 2 tablets for each cup of tea she drinks. Considering that she likes tea and drinks about 3-5 cups daily, I had to take a minimum of 3 tablets and max of 15 to see toxic effects of increased concentrations. As the sufficient watering period for *P. Oleracea* is once in 3-7 days[7] I took an average of 5 days period and watered the plants with defined numbers of dissolved aspartame in distilled water. While dissolving the aspartame tablets in water, I used a stirrer to dissolve it until no visible particles left. Although, I tried my best to perform this there may be some sedimentation on the bottom of the cup. So I used different cups in order to avoid any change in the dissolved amount of aspartame in solution and washed each cup after and before each use.

[7] http://hbogm.meb.gov.tr/modulerprogramlar/kursprogramlari/bahcecilik/moduller/domates_yetistiriciligi.pdf

My aim was to determine the toxic effect of aspartame on a living organism. While I was measuring the length and number of leaves of the plant to indicate its health, I also looked for other qualitative changes. During my research before the experiment I found out that plants can have cancer too. *Agrobacterium tumefaciens* is the bacterium which causes tumours to appear on the stems of plants. It causes tumours by transferring genes to the cells of the infected plant cells from a tumour inducing plasmid. The transferred DNA is integrated essentially randomly (no apparent sequence bias at the site of insertion) into the plant chromosomes and normally add bacterial genes that stimulate plant tumour cell growth.[8] One of the symptoms of cancerous plants is having white dots on leaves. I surprised a little bit when I first saw the dots on my plant samples. I was checking daily for it; however, I wasn't expecting to actually see them. The conditions of the plants are checked daily and it is found that there are no dots on the sample which is watered without any aspartame. So I will use it as an indicator of the health of the plant and toxic effects of aspartame.

[8] http://www.i-sis.org.uk/Agrobacterium.php

MATERIALS USED IN EXPERIMENT

1. 25 identical plastic flowerpot (700 ml)
2. 125 identical *P. Oleracea* plants
3. Loam Soil (sufficient)
4. 5 Graduated cup (200 ml)
5. 5 Stirrers
6. 1500 aspartame tablets (Sanpa©) (20 mg each)
7. Distilled water (22 Liters sufficient)
8. Thermometer
9. Ruler (30cm)
10. NaOH (Merck©, Germany) (0.1M in sufficient amount)
11. Electronic scale
12. pH meter (Orion©, USA)
13. Dropper (100 ml)

IV. METHOD

1. Several *P. Oleracea* saplings at same age and quality are planted to identical pots and cultivated for 15 days
2. Twenty five *P. Oleracea* were chosen among the 1st cultivated ones which were 6 cm in height (measured with a ruler) and having exactly 12 leaves each.
3. Identical pots are placed carefully near window in order to control the sunlight intensity
4. Five *P. Oleracea* are planted in each 5 pot filled with loam soil taken from the same package. Humidity, temperature and pressure are kept constant by keeping all pots in the same room
5. 120 ml distilled water is added to the first pot without adding any aspartame in order to observe a normal sample.
6. 3 aspartame tablets are dissolved in 120 ml of water with a stirrer in 22°C.
7. Sufficient 0.1M NaOH is added to the solution with a dropper in order to keep the pH level equals to 7 and a total of 120 ml solution is obtained, pH is measured by a pH meter.
8. The solution is poured into the pot of plant. This watering process is repeated once in 5 days for a period of 20 days totally.
9. Repeat steps 6-8 for 6, 10 and 15 aspartame tablets in 120 ml of water.
10. Lengths of the plants are recorded after each watering. Physical changes like number of leaves noted down
11. Perform another 4 independent trials (a total of 5 trials)

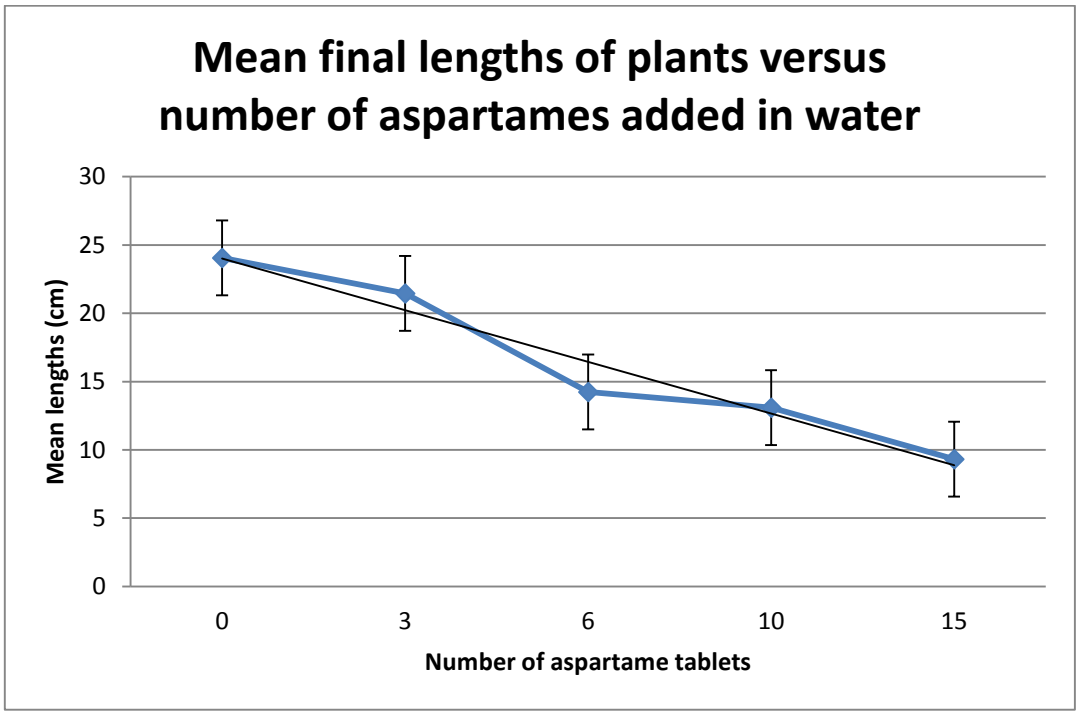
Number of Aspartame tablets added	Trial	Final length (±0.5cm)	Initial length (±0.5cm)	Final Number of Leaves	Initial number of leaves	Growth time (days)	Volume of aspartame solution (±0.5ml)	Temperature of solution (±0.5 °C)	Atmospheric Pressure (±0.5 mmHg)
0	1	23.2	6.0	16	12	20.0	12.0	22.0	1076.0
	2	23.6		18					
	3	24.0		16					
	4	25.7		19					
	5	23.8		16					
3	1	20.6		10					
	2	21.0		12					
	3	22.0		10					
	4	22.3		13					
	5	21.4		10					
6	1	14.0		7					
	2	14.2		9					
	3	14.4		8					
	4	14.4		8					
	5	14.2		6					
10	1	12.8		4					
	2	13.0		5					
	3	13.0		3					
	4	13.5		3					
	5	13.2		4					
15	1	10.1		1					
	2	8.4		2					
	3	9.0		1					
	4	10.3		0					
	5	8.8		1					

Table 1: The data obtained from the *P. Oleracea* samples after 20 days of growth watered by different concentrations of aspartame solution at constant volume of tap water, temperature of the solution, light source, initial length, number of leaves and mass of plant used in each trial, and pressure

V. RESULTS

	0	3	6	10	15
Mean of Length	24.1	21.5	14.2	13.1	9.3
Median	23.8	21.4	14.2	13.0	9.0
Range	2.5	1.7	0.4	0.7	1.9
Variance	0.9	0.5	0.0	0.1	0.7
SD	1.0	0.7	0.2	0.3	0.8
SE	0.4	0.3	0.1	0.1	0.4
T	2.8	2.8	2.8	2.8	2.8
95%CI(TxSE(0.05))	1.2	0.9	0.2	0.3	1.0

Table 2: This table shows the results of specific calculations made on gained data from length of the plants cultivated with different concentrations of aspartame for 20 days.



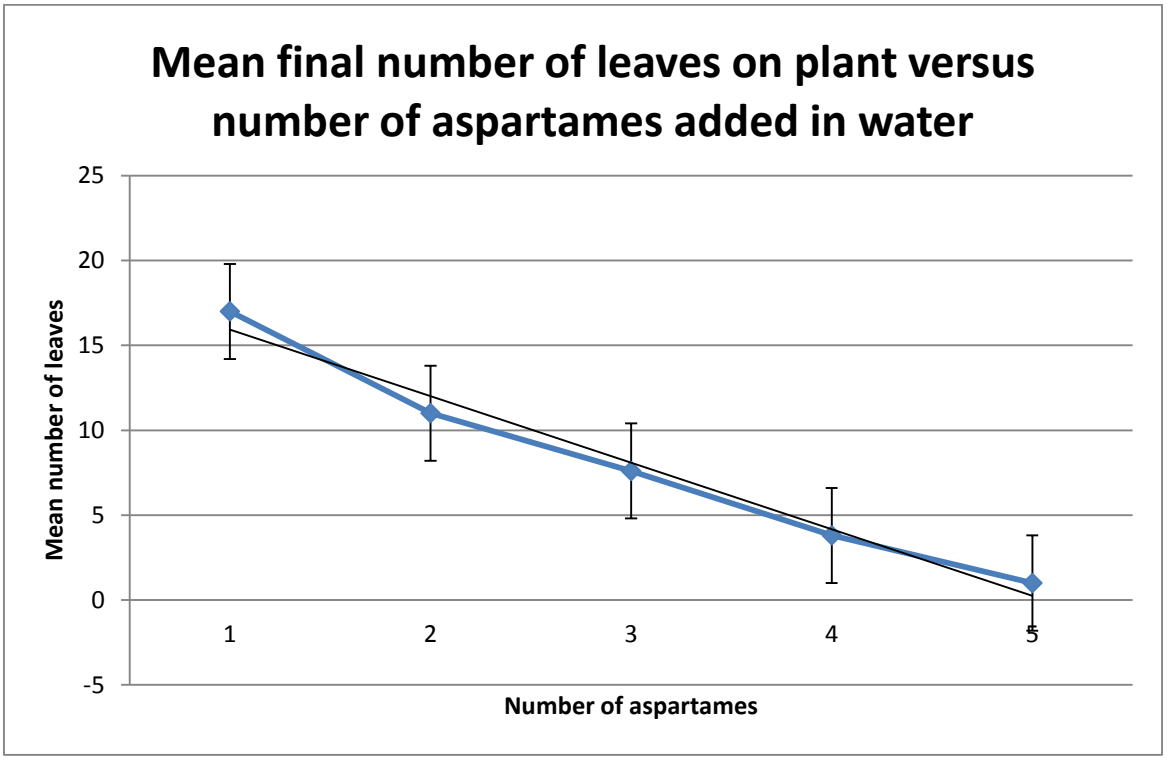
Graph 1: Mean values of final lenghts of plants after 20 days versus number of aspartame tablets used in watering with best fit line and error bars. (Error bars are based on standard error)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
0	5	120.3	24.06	0.928		
3	5	107.3	21.46	0.488		
6	5	71.2	14.24	0.028		
10	5	65.5	13.1	0.07		
15	5	46.6	9.32	0.697		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*
Between Groups	749.7736	4	187.4434	423.8883	5.0469E-19	2.866081
Within Groups	8.844	20	0.4422			
Total	758.6176	24				

Table 3: The Anova single factor table of the collected data on length of plants cultivated with different numbers of aspartame tablets for 20 days.

	0	**3**	**6**	**10**	**15**
Mean Number of Leaves	17.0	11.0	7.6	3.8	1.0
Median	16.0	10.0	8.0	4.0	1.0
Range	3.0	3.0	3.0	2.0	2.0
Variance	2.0	2.0	1.3	0.7	0.5
SD	1.4	1.4	1.1	0.8	0.7
SE	0.6	0.6	0.5	0.4	0.3
T	2.8	2.8	2.8	2.8	2.8
95%CI(TxSE(0.05))	1.8	1.8	1.4	1.0	0.9

Table 4: This table shows the results of specific calculations made on gained data from leaves of the plants cultivated with different numbers of aspartame tablets for 20 days.



Graph 2: Mean values of final leaf numbers of plants after 20 days versus number of aspartame tablets used in watering with best fit line and error bars. (Error bars are based on standard error)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
0	5	85	17	2		
3	5	55	11	2		
6	5	38	7.6	1.3		
10	5	19	3.8	0.7		
15	5	5	1	0.5		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	783.84	4	195.96	150.7385	1.24E-14	2.866081
Within Groups	26	20	1.3			
Total	809.84	24				

Table 5: The Anova single factor table of the collected data on number of leaves of plants

Number of Aspartame added	Trial	Lifetime (days)	pH level
0	1	49	7.0
	2	48	
	3	48	
	4	47	
	5	48	
3	1	44	
	2	43	
	3	45	
	4	44	
	5	44	
6	1	37	
	2	36	
	3	35	
	4	37	
	5	37	
10	1	25	
	2	27	
	3	25	
	4	26	
	5	26	
15	1	20	
	2	20	
	3	19	
	4	21	
	5	20	

Table 5: The data obtained from the *P. Oleracea* samples' lifetimes, watered by different concentrations of aspartame solution lifetime of the plants and the pH level through the experiment.

VI. CONCLUSION AND EVALUATION

In this experiment, the effect of aspartame sweetener tablet is investigated on the growth of the plant Purslane, *P. Oleracea.* The samples of plants are taken from the same root and placed carefully in a pot before filling with soil. The soil used is also taken from the same package and filled the pots in same volume as the pots are identical. To ensure the growth of the plant, the samples are placed under sunlight with a location that allows each to take the same light intensity. The plants are chosen to have the same length, number of leaves and mass to obtain more precise data from identical samples. Although, being in the same room, to keep the temperature and pressure same for all samples thermometers are used for each pots to make sure that there is no difference.

Those pots are watered once in 5 day with different numbers of aspartame tablets dissolved in water. To observe the effects of the aspartame on the growth of the plant, change in length and leaf numbers were noted down daily. All of the tablets were taken from same package of Sanpa© aspartame made under same factory conditions, so each tablet assumed to be completely identical.

The hypothesis stated before was saying that the increasing amount of dissolved aspartame in watering solution will decrease the rate of growth and number of leaves plant have.

The statistical analysis made on the obtained data supports my hypothesis as the P-value given by Anova Single Factor[9] test is less than the alpha value 0.05 for each variable (See tables 3 and 5). The resultant P values are 5.0469E-19 for length of the plant and 1.24E-14 for number of leaves on the plant respectively. Obtained P-value is less than alpha value which proves that the hypothesis made before is true and can to be accepted with 95% confidence.

[9] http://www.graphpad.com/support/faqid/782/

The truth of the hypothesis can be also proved by checking the mean values of the lengths and leaves. As the mean final lengths of the plants were 24.1 (see table 2) with no aspartame addition and 9.3 with 15 aspartame tablets dissolved in water are obtained. It can be said that the maximum final length is observed in watering without aspartame and minimum in 15 aspartame dissolved in water. Also maximum number of leaves is reached with no aspartame added plant which is 17 and minimum is 1 which is watered with solution containing 15 aspartame tablets (see table 4). Based on these quantitative data, it is clear that using aspartame effects the growth of the plant.

In order to validate the results by statistical way, standard error and standard deviation values evaluated. When the standard error values for both of the variables length and leaf numbers are checked, it can be seen that they are low. The highest one is 0.6 for leaves and 0.4 for lengths and lowest values are 0.3 and 0.1 respectively (see table 2 and 4). Less standard error means that the result I got is accurate.

I also checked the standard deviation values in order check the precision of my data. It has low values for both of the variables so that it can be said that my data are precise too. Highest SD value for leaves is 1.4 and lowest is 0.7. It is 1.0 0.2 respectively for the length of the plants. (see table 2 and 4).

I also kept the experiment running until the plants die for further investigation. The effect of aspartame on the lifetime of the plant can clearly be stated as it shortens the lifetime with each increasing amount in aspartame addition. (see table 5).

During the experiment I have seen several dots only on the leaves of plants that were fed with water containing aspartame. This phenomenon also proves the negative effects of aspartame on the health of *P. Oleracea* (see figure 1)

Figure 1: The qualitative observations made on the plant. White dots appeared on leaves of aspartame added *P. Oleracea* plants.

Even though all these quantitative proofs there are still errors causes the experiment not to be trusted 100%. In the very first trial, I poured the solutions to pots from the same cup each time. After realizing that white dots appeared even on the no aspartame added plant I decided to start over and care on the pots next time. So that the main experiment was held with different cups and washed before and after every use. Like that, I tried to keep the constants controlled throughout the experiment, however, some of them were hard to keep controlled and some can be improved:

First of all, it doesn't matter how hard I tried to take the plants identical. Even taking them from the same root does not make them identical. So there could be differences in the growth of the plants.

Aspartame tablets have ingredients. Which means that they do not contain pure aspartame in it. Other ingredients in the tablets might cause a change in plants' growth.

The solution used in watering was not distilled completely. Also the soil used can have different minerals in it which will effect the growth of plants.

Suggestions:

In order to clarify the results (if we can't make a copy of the same plant) there should be more trials held and the range of aspartame tablets should be widened. So that the error caused by the difference of the plants can be minimized.

Pure aspartame could be used to investigate the toxic efects of aspartame. By adding pure aspartame to water we can omit the effects of other ingridients formulated in Sanpa© tablets that may have other toxic effects on the plant. (see appendix 1)

As a total solution the experiment could be held in a greenhouse to stabilize the environmental conditions such as light, temperature, pressure and humidity.

A technic called hydroponic farming (see appendix 2) which will omit the effects of soil in the experiment and only consider water. By this way; the minerals and nutrients, that plants able to get, can be controlled easily.

Results of this experiment would be more accurate and reliable if I could use yeast cells. Because those cells are eukaryotic and share many similar metabolic pathways with human cells.

I could also test the toxic effects of aspartame by performing cell culture, especially using human cells. By this way different and more reliable results may have been obtained.

In this research I could only observe the phenotypic characteristics of plants. Investigating the genotypic changes may give more effective results concerning any geno-toxic effects of aspartame.

It would be more useful, if I could test the toxic effects of other artificial sweeteners and compare their effects with that of aspartame.

To sum up I strongly advise the aspartame users to change their artificial sweetener with natural sweeteners. As this experiment proved them the side effects of aspartame on a living organism. Even the diabetic people who should use it to asist their treatment, at leat have to decrease the amount of aspartame they are taking, if they can not totally stop using them.

I am very happy that I did a research related to the toxic effects of aspartame. Because at the end of this research, my mother stopped using aspartam tablets. Now she is trying to drink her tea without any sweeteners.

APPENDIX

Appendix 1:

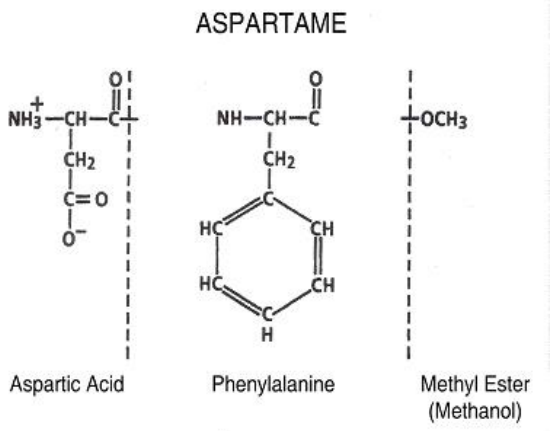


Figure 2: The chemical structure of aspartame.

Created from aspartic acid, phenylalanine and methyl ester. Phenylalanine must be avoided by people with phenylketonuria (PKU), a rare genetic condition.[10] Those extra ingredients can cause different effects on a eukaryotic cell.

[10] http://www.befoodsmart.com/ingredients/aspartame.php

Appendix 2:

Figure 3: A greenhouse using hyroponic farming method

Hydroponics is a farming method of growing plants inside an enclosed structure without soil, but in a selected growing medium where the lighting, temperature, and nutrients are closely regulated.

There are two basic forms of hydroponics: soil-less mediums, such as clay, sand, rock wool, perlite, and vermiculite, and true hydroponics, which use only a water-based solution of required plant nutrients. The most commonly grown hydroponic crops in the United States are lettuce, tomatoes, peppers, cucumbers, herbs, and flowers.

Soil research experts discovered that soil acts as a stabilizer, keeping the plant in a vertical growing position, and as a source of minerals for the plant. Experiments with flowers and vegetables suggest that if a plant's structure is given artificial support, its root system can absorb all of the nutrients it needs directly from a water-based solution without the need for soil.[11]

[11] http://www.naturalstandard.com/demo/demo-eg-hydroponicfarming.asp

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