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The Amount of Water and Sunlight for Growth of *Stevia Rebaudiana*: Input to Propagation Program

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This study was developed to propagate a kind of food sweetener that is a product of a nature-blessed plant known as Stevia rebaudiana, a plant species in the genus Stevia of the sunflower family (Asteraceae), commonly known as sweet leaf, or sugar leaf which originated from the highlands of Paraguay, South America.

Stevia rebaudiana has been recognized in various studies to have properties that provide organic sweeteners which can be helpful to diabetic patients. It is now recognized as a healthy sugar substitute not only for those suffering with diabetes but also for health-conscious individuals (Hannah Nichols, 2018).

Since the plant can be adopted in a warm country like the Philippines, it is very vital to raise this rare kind of plant to lessen the problem of sweetener shortage and to promote healthy kind of eating practice.

Two experimental groups of five (5) plant samples were investigated on the amount of water and amount of sun exposure combination (ratio) designated as: a) 1:1 – minimum water and minimum sunlight; b) 1:3 – minimum water and moderate sunlight; c) 2:2 – medium water and medium sunlight; d) 3:1 – maximum water and minimum sunlight; and e) 3:4 – maximum water and maximum sunlight.

The results obtained from both experimental groups showed that the plant sample investigated under the 1:3 combination (minimum water and moderate amount of sunlight condition) posted the highest growth while the plants exposed under the 3:4 combination (maximum water and maximum sunlight) were observed to have achieved the shortest growth. The stevia plants grew best when exposed to minimum amount of water and moderate sunlight.

Keywords: stevia, water. Sunlight, propagation

Introduction

Medicinal plants nowadays are not just used by pharmaceutical companies as major components in medicine production to treat human diseases. According to Okwu (2001), many indigenous medicinal plants are also used as spices and foods. The Philippines, among other Asian nations, has a rich biodiversity of such plants and different universities involved in agriculture are currently in the process of conducting studies to document the nutritive/medicinal values of less familiar plant species. *Stevia rebudiana* is slowly being known in the Philippines because it is believed that it could be a good source of organic sugar substitute for diabetic patients. Since the plant is not native to the country, there is a necessity to conduct a study on how to propagate the plant in order to benefit from it.

Stevia rebaudiana has been recognized in various studies to have properties that provide organic sweeteners which can be helpful to diabetic patients. It is now recognized as a healthy sugar substitute not only for those suffering with diabetes but also for health-conscious individuals. With the increasing awareness all over the world to turn organic especially in food and medicine production for health reasons, it is imperative that a study be conducted in line with the propagation of *Stevia rebaudiana.*

Stevia rebaudiana (Bertoni) commonly known as sweet leaf is a perennial shrub and is a member of *Asteraceae* family. According to Cramer and Ikan (1987) the plant is native in the valley of the Rio Monday in highlands of Paraguay between 25 and 26 degrees south latitude where it grows in sandy soils near streams. It was first consumed over 200 years ago in South America where the indigenous people used the leaves of the plant to sweeten beverages or chewed them for their sweet taste. Traditionally, the plant leaves, often called 'sweet herb', were dried and used to sweeten maté, teas and medicines. Moreover, Cramer and Ikan (1987) noted that it is also being cultivated in some regions of Asia, Europe and Canada.

The *Stevia* plant was first scientifically recorded in 1899 as *Eupatorium rebaudianum* by Moises Santiago de Bertoni in Paraguay. In 1905, it was later defined as *Stevia Rebaudiana,* a member of the sunflower (*Asteraceae*) family.

Stevia was first commercially adopted as a sweetener in the 1970s in Japan where it is still a popular ingredient today. Stevia is cultivated mostly in Paraguay, Kenya, China and the United States, and within many other parts of the world, including Vietnam, Brazil, India, Argentina and Colombia.

The principles of *S. rebaudiana* are due to natural sweet active components present in the leaves that are stevioside and rebaudiosides A, B, C, D, and E; dulcoside A; and steviolbioside. Stevioside has a slight bitter aftertaste and provides 250–300 times the sweetness of sugar. The sweet diterpenoid glycoside, rebaudioside F, has been isolated from leaves and its structure was established by chemical and spectral studies.

Manish Tadhani and Rema Subbash (2006), in a scientific paper, investigated the proximal composition, mineral analysis and phytochemical screening of the *Stevia rebaudiana* leaves. The study also investigated the fatty acid composition of the *Stevia* leaf oil. The results showed that protein, carbohydrate and ash content of the *Stevia* leaf was found to be higher whereas fat was estimated to be less in the leaf on dry weight. The mineral analysis on the same study showed the presence of high amount of potassium, calcium, magnesium, phosphorous, sodium and sulphur content while iron, manganese, zinc, copper, molybdenum, selenium and cobalt content were found out to be at ppm levels on dry weight basis.

The same study subjected powdered *Stevia* leaves to preliminary phytochemical screening using chemical method and found out that the most abundant compound in the leaf extract were tannins and alkaloids, followed by cardiac glycosides, saponins, sterols, and triter penes reducing compounds and anthraquinones. There was a negative result, however, when test for cyanogenetic glycosides was performed.

Fatty acid analysis performed in the same study identified the presence of palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid in the leaf oil. Palmitic acid content was found to be highest whereas stearic acid was found to be least among the identified fatty acids.

Shruti Shukla et.al (2013), in a study conducted on the phytochemical screening and anthelmintic and antifungal activities of leaf extracts of *Stevia rebaudiana,* concluded that the traditional use of the leaves of *Stevia rebaudiana* as an anthelmintic was confirmed when the aqueous and ethanolic leaf

extracts displayed activity against the worms used (*B. cinerea* and *F. oxysporum*) in the study. Dunti Shukla added that the antifungal effects of both extracts of *Stevia rebaudiana* against plant pathogenic fungi may offer new applications to the agricultural industry as an alternative to synthetic chemical fungicide for their possible antifungal applications.

In the United States, *Stevia* is mostly employed as sugar substitute. About one-fourth teaspoon of the natural ground leaves is equivalent to one teaspoon of sugar. In South America, a standard infusion is sometimes used as a natural aid for diabetes and hypertension. The difference between *Stevia* and sugar is that the former does not cause tooth decay. It has been reported that *Stevia* kills the bacteria *Streptococcus mutans*, which is the prime factor in teeth plaque.

Active principles of many plant species are isolated for direct use as drugs, lead compounds, or pharmacological agents. Different species of medicinal plants are used in the treatment of diabetes mellitus. For diabetes treatment, before the discovery of insulin, the only options were those based on traditional practice.

Crammer and Ikan, (1987) in their study entitled "Progress in the Chemistry and Properties of the Rebaudiosides in the Developments in Sweeteners" stated that the plant which is indigenous to Paraguay has showcased medicinal properties. Its medicinal use includes regulating blood sugar, preventing hypertension, treating skin disorder, and preventing tooth decay. It also possesses antibacterial and antiviral properties. Standard extracts of *S. rebaudiana* are used as natural sweetener or dietary supplements in different countries for their content of stevioside or rebaudioside A. These compounds possess up to 250 times the sweetness intensity of sucrose and they are non-calorigenic.

Chan et al. (2000) together with Lee et al. stated that the *Stevia* extracts have beneficial effects to human health as antihypertensive. On the other hand, Jeppensen et al. (2000) suggested its antihyperglycemic effect on the human body. Takeshi et al. (2001) found out that the *Stevia* extracts have antihuman rota virus activities while Das et al. (1992) in their study found out that it is noncariogenic. *Steviza* extracts are used as sweeteners and are thought to influence glucose metabolism (Suanarunsawat & Chaiyabutr, 1997)

Statement of the Problem

The general problem of this research is: How may *Stevia Rebaudiana* be propagated in large quantities? Specifically, it sought to answer the question: What amount of water and exposure to sunlight are needed to obtain maximum growth in a prescribed period of time?

Materials and Methods

This study which is experimental in nature commenced with a benchmarking activity about the *Stevia rebaudiana* at Central Luzon State University. The interview type of gathering raw data was utilized using an interview guide question drafted by the researcher and later on improved by the Bulacan State University (BulSU) researcher.

The first experimental design made use of five pairs of plant samples. Two pairs were placed under the direct heat of the sun for a whole day, two pairs were placed under the same condition for four hours and were afterwards taken into the shade. One pair was placed under the shade for a whole day while the other two pairs were placed inside the house. Water was set at 100 ml each, for the first pair, 80 ml for the second pair, 60 for the third pair and 40ml and 20 ml for the last two pairs of plant samples. After four days the plant samples placed under the sun dried up while the other pairs of samples slowly dried. This resulted in a formulation of another experimental design.

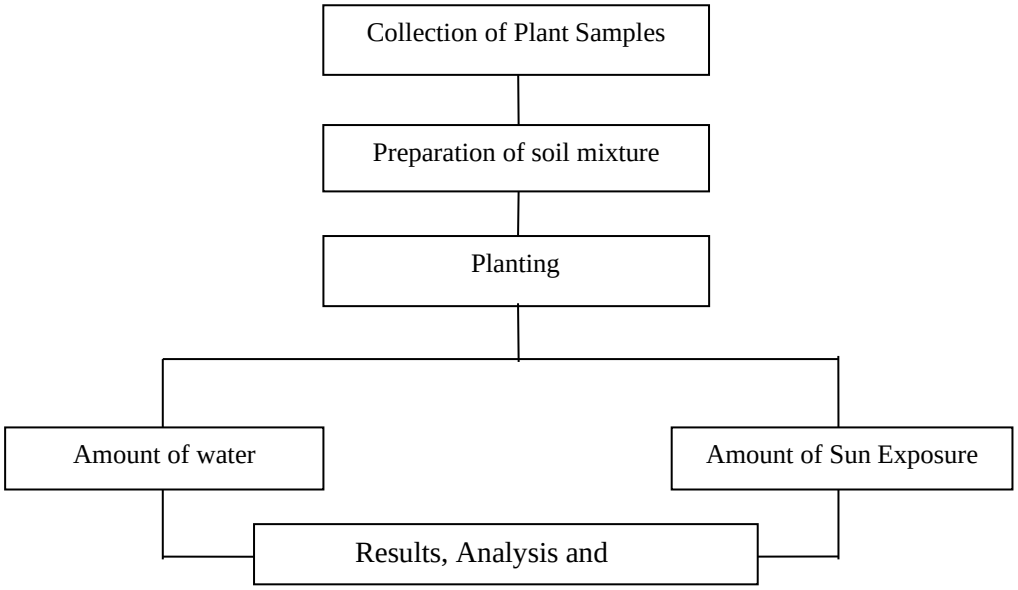

Figure 1. Flow Chart of Activities

Figure 1 illustrates the activities for the second experimental design. Collection of the plant samples (specimen) was done by purchasing *Stevia rebaudiana* seedlings from the EDSA Plants Center in Quezon City. Soil mixture considered as the controlled variable was prepared and was followed by planting the plants specimen using the soil mixture. Plants were cut from the roots of the seedlings and then planted to the soil mixture. Amount of water and amount of sun exposure combination were formulated in order to determine the growth of *Stevia rebaudiana* Results analysis and conclusions were drawn after a thorough observation of the plant samples was conducted.

Five pairs of plant samples were also used in the second experimental design. The amount of water was labeled as low (10 ml), medium (20 ml) and high (30 ml), while 25 watts, 40 watts, 60 watts and 100 watts incandescent lamps were utilized in order to determine the effect of sun exposure to each pair of plant samples. The plants were watered in the morning and were placed under the 25, 40, 60 and 100 watt bulbs for three hours each day. The plant samples were placed outside at night to allow them to catch the morning dew.

Table 1 presents the soil materials content per pot of soil mixture used in planting the *Stevia rebaudiana* seedlings. With the exception of Bio char, which weighed only 50 grams per pot, all the other soil materials weighed 100 grams dry weight.

Table 1. Soil Mixture per Pot

Soil materials	Content per pot
Garden soil	100 grams dry weight
Perlite	100 grams dry weight
Carabao manure	100 grams dry weight
Coir (grounded coconut husk)	100 grams dry weight
Bio Char (pulverized charcoal)	50 grams dry weight
Total	**450 grams dry weight**

Table 1 shows the soil mixture consisting of garden soil, perlite, carabao manure, coir (grounded coconut husk) and Bio char (pulverized charcoal) placed onto the pots prior to the planting of the *Stevia rebaudiana* seedlings.

Shown on Table 2 is the description of the amount of sun exposure of the *Stevia rebaudiana* seedlings in terms of watts of incandescent lamps. Plants placed under this experimental condition combined with the prescribed amount of water were classified to be under low sun exposure when placed under a 25-watt incandescent bulb.

The 40-watt incandescent bulb was considered to supply a medium amount of sunlight over a plant sample. The 60-watts and 100-watt incandescent light bulbs were described to be under moderate, and high sun exposure, respectively. The table also shows how many lumens were generated by the identified watt units of the incandescent light bulbs.

Table 2 also describes the volume of water utilized in the experimental conditions of the study. The 30 ml volume of water used in watering the *Stevia* seedlings daily was described as high, the 20 ml volume used in the same experiment for the prescribed combination of water and sun exposure was described as medium and the 10 ml volume used was described as low.

Table 2. Descriptive Measures of the Amount of Sun Exposure and Amount of Water

Amount of light in Watts (lumens)	Description
1. 25 watts (375 lm)	Low
2. 40 watts (600 lm)	Medium
3. 60 watts (900 lm)	Moderate
4. 100 watts (1500 lm)	High
Volume of Water	**Description**
1 10 ml.	Low
2. 20 ml.	medium
3. 30 ml.	high

These prescribed wattages (lumens) of incandescent light bulbs simulate the plants' sun exposure under controlled conditions. Each bulb was combined with the desired amount of water to determine the effects of the amount of water and sun exposure on the growth of *Stevia reabaudiana.*

Table 3. Descriptive Measures of Water and Sunlight Combination on Plant Samples

Plant Sample	Water and Sunlight combination	Description (water: exposure to light)
1. Plant A	1 - 1	low - low (10 ml: 25 watts)
2. Plant B	1 - 3	Low - moderate (10 ml : 60 watts)
3. Plant C	2 - 2	Medium – medium (20 ml:40 watts)
4. Plant D	3 – 1	High – low (30 ml: 25 watts)
5 Plant E	3 - 4	High-high (30 ml: 100 watts)

Table 3 indicates the amount of water and the amount of sunlight exposure employed in each of the plant samples. Plant A was observed under the 1:1 amount of water and amount of sunlight condition. Under this experimental condition, the plant sample designated as plant A received 10 ml water daily under the heat of a 25-watt incandescent lamp.

Another plant sample designated as plant B was investigated under the experimental condition set forth by the 1:3 ratio of 10 ml water and 60-watt incandescent light bulb. On the other hand, the plant which was investigated under the combination of 20 ml water and 40-watt bulb was classified as plant C with a 2:2 ratio. Experimental condition set by the 3:1 ratio employed 30 ml of water and 25-watt bulb on plant D. The last plant classified as plant E was observed under the 3:4 water sunlight ratio of 30 ml water and 100-watt incandescent lamp.

The experimental conditions for amount of water and amount of sunlight were employed on two sets (Groups 1 and 2) of *Stevia rebaudiana* plant samples. Both sets of samples were observed in this study.

Results and Discussion

Table 4. Growth in Millimeters (Experimental Group 1)

Day	Plant A (1-1)	Plant B (1-3)	Plant C (2-2)	Plant D (3-1)	Plant E (3-4)
1	0	0	0	0	0
2	0	0	0	0	0
3	3	4	3	2	0
4	3	7	5	4	0
5	4	11	9	6	0
6	5	17	14	9	1
7	6	22	20	12	2
8	7	28	25	14	3
9	9	34	34	18	4
10	10	41	38	21	5
11	15	48	44	25	6
12	20	55	49	31	7
13	20	61	53	32	10
14	23.5	66	54	32	12
15	27	72	56	35	13

Table 4 shows the growth in millimeters of the *Stevia rebaudiana* experimental group 1 from Day 1 to 15. All the plants under observation did not register any growth for the first two days.

Plant growth was recorded on the third day with the exception to plant E which did not post any growth until day 6. Plant E posted a steady minimal growth which reached only 13 mm in a span of fifteen days. Highest growth level was obtained by plant B with a total growth of 72 mm at day 15. Plant C on the other hand recorded a 54 mm growth on the last day while plants D and A registered growth of 35 mm and 27 mm, respectively. On day 9, both plants B and C registered the same growth at 27 mm each.

It can be noted that while plant samples A to D posted steady increase growth level from day 3 onwards, plant E exhibited 1 mm growth on day 6. This 1 mm growth continued up to day 12. On day 13, it posted a 3 mm growth, and a 2mm growth on day 14, until it reached a total of 13 mm increase on day 15.

Table 5. Growth in Millimeters (Experimental Group 2)

Day	Plant A (1-1)	Plant B (1-3)	Plant C (2-2)	Plant D (3-1)	Plant E (3-4)
1	0	0	0	0	0
2	0	0	0	0	0
3	4	1.5	2.5	4	0
4	4.5	3.5	4	5	0
5	6	7.5	6	7	0
6	7.5	11	9	10	1
7	9	17	15	13	1.5
8	10.5	22	21	18	2.1
9	12	27	27	21	2.5
10	13.5	34	32	25	3
11	15	42	36	29	3
12	16.5	49	41	34	3.5
13	17.5	56	44	36	3.7
14	20.2	59	45	39	4.5
15	27.7	64	49	41.5	5.5

Table 5 exhibits the growth (in millimeters) of plants under experimental group 2. The plants in this group were also observed under the same experimental conditions employed in experimental group 1. Plant E did not register an increase in height until day 6 where it posted only a 1 mm growth while all the other plant samples were observed to have an increase in height on the third day of observation. Moreover, plant E manifested a very slow increase in height. It could be noted that the level of growth by sample E was all less than 1 mm daily from day 6 up to day 14. It posted a 1 mm increase only on day 15. Plants A to D grew up steadily at varying rates from day three up until day fifteen.

Among the plant samples in this group (2), plant B was recorded to achieve a 64 mm growth on the last day of observation. This was followed by Plant C with 49 mm growth. Plants D and A recorded 41.5 mm growth and 27.7 mm growth, respectively. The highest increase in growth was attributed to

Plant B with experimental condition of low amount of water and moderate amount of sun exposure. The slowest growing plant was Plant E with a total growth of 5.5 in fifteen days.

The data generated after observation of the two experimental groups show that plant E with a water-sunlight ratio of 3: 4 (high amount of water and high rate of sunlight exposure) posted a very slow growth. *Stevia* plants with water sunlight ratio of 1:1 (plant A) (low amount of water (10 ml) and low amount of sun exposure (25 watts)) had a slight difference in the total height achieved during the fifteen-day period. Difference was recorded at 0.17 mm between experimental group 1 and 2. Plant A under minimum conditions both grew steadily and achieved the same height at day 11, but plant sample in group 1 did not grow between days 12 and 13 as recorded by the same height achieved for two days. On the other hand, the same plant (A) in experimental group 2 continued to grow steadily until it suddenly increased in growth from 20 mm to 27.7 mm from day 14 to 15. Moreover, it was found to be the second slowest growing plant sample.

Data on experimental group 1 show that plant B under 1:3 ratio (10 ml water and 80-watt electric bulb) achieved a 72 mm growth which is the highest recorded growth. On the other hand, plant C (2:2 ratio) investigated at medium amount of water and medium amount of sunlight was found out to have grown by 56 mm which placed second in the experimental group. This result was affirmed by the results on experimental group 2 (see Table 6), which recorded a 64 mm growth by plant B. This was followed closely by a 49 mm growth in plant C. Because of the similarities in the findings of both experimental groups, it could safely be said under the same experimental conditions employed on the two experimental groups, the 1:3 water and sun exposure ratio manifested the highest increase in growth of *Stevia rebaudiana*.

Plant D (3:1 ratio) with high amount of water and moderate amount of sun exposure was ranked third in the increase in growth. In the first experimental group, it manifested a total growth of 35 mm on day 15 while in experimental group 2 its height was measured at 41.5 mm.

Based on the above findings, it is safe to conclude that *Stevia rebaudiana* grows best under low amount of water and moderate amount of sun exposure. This finding supports the claim of Dr. Lucena B Gajete, an agriculturist with specialization in crop protection at Central Luzon State University, that states that the *Stevia* plants grow best under partial shading. According to Dr. Gajete, shading requirement for *Stevia rebaudiana* ranges from 40-50%. The findings for the amount of water further support the claim of Dr. Gajete that *Stevia rebaudiana* grows best in well-drained soil since water clogging the roots dry up the plant. This finding also confirms the result on the article on Mother Earth News which says that the plant grows very well in warm climates and well-drained soil.

Conclusion

After analyzing the results of this experiment, the following conclusions were drawn:

1. *Stevia rebaudiana* plants grow best under minimum water and moderate exposure to light (1:3) with plant B samples from experimental groups 1 and 2 registering maximum growth 72 mm and 64 mm, respectively.

2. The application of a medium water and medium amount of sunlight (2:2) combination on *Stevia rebaudiana* seedlings also yields a desirable growth.

Recommendations

Since the study was limited only to the water and sunlight combinations, it is recommended that a follow up study be made which will include the number of fertilizers to be used. Moreover, it is proposed that the follow up study employing the spreading *Stevia rebaudiana* seeds on the soil must be conducted since this kind of method was not included in this study.

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