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# Growth and productivity of stevia variety Cibodas Manis 3 (*Stevia rebaudiana*) in 40 days planting with agroforestry

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**Abstract.** *Choeriah MN, Wijayanto N, Batubara I. 2024. Growth and productivity of stevia variety Cibodas Manis 3 (*Stevia rebaudiana*) in 40 days planting with agroforestry. Biodiversitas 25: 3277-3282.* Stevia (*Stevia rebaudiana* Bertoni.) is a shrub plant that can be used as a substitute for sugar cane and has the potential to be cultivated in agroforestry ways. This study aims to analyze the growth and productivity of stevia in agroforestry systems compared to monocultures. Stevia was planted in 2 types of land, namely agroforestry and monoculture. The research location's soil and area's climatic conditions were observed stevia agroforestry was grown under pine, eucalyptus, and coffee trees. During stevia planting, the height was measured. Stevia was harvested 40 days after planting, and leaf area, number of leaves, leaf weight, and harvest productivity were measured. In addition, chlorophyll and stevioside yields were analyzed using UV VIS and LC-MS/MS spectrometry. Stevia in agroforestry land had higher chlorophyll and stevioside content than monoculture, although not as good as stevia in monoculture, which had higher height, number of leaves, leaf area, wet weight, dry weight, and productivity compared to agroforestry. The total stevioside produced from agroforestry land was 1.63 kg/ha, while it was 2.19 kg/ha from monoculture land. Stevia in agroforestry land also has a higher secondary metabolite content than in monoculture. This is related to the growing conditions that stress stevia. Pearson correlation test results also show a relationship between the amount of chlorophyll and the stevioside content in stevia plants.

**Keywords:** Agroforestry, light intensity, secondary metabolite, stevia, stress

## INTRODUCTION

The issue of deforestation has now become a global issue that requires control and prevention (Khuc et al. 2020). Agriculture is one of the causes of deforestation and changes in forest land (Gingrich et al. 2022), including sugarcane farming for sugar production. According to the USDA (2022), sugar consumption as a sweetener is increasing in several countries. The Natural History Museum (2021) reported several cases of forest and environmental damage due to converting forest land to sugarcane plantations. For example, in Brazil, sugar cane farms have been the biggest driver of deforestation, and they are particularly damaging to rainforest areas because they use a lot of water to produce.

One way to produce sweetener crops without damaging land is through agroforestry. It is a sustainable land-use system that integrates trees with seasonal crops, farms, and other agricultural activities to initiate agroecological succession and increase ecologically and economically values. Agroforestry is one of the land processing systems that can be used to solve the problems arising from the existence of land use and, at the same time, address food shortage problems. Agroforestry aims to increase land productivity, prevent increasing degraded land, preserve forest resources, improve agricultural quality, better intensify and diversify forestry, create jobs, and increase rural communities' incomes (FAO 2022).

Stevia (*Stevia rebaudiana* Bertoni) is a spruce plant that can be used as a sugar substitute for cabbage (Sharma et al. 2015). Stevia is a crop that can potentially be grown in the agroforestry system. Furthermore, stevia agroforestry is expected to be a solution in reducing the forest land into monoculture agricultural land changes. Stevia's fertility level is 300 times that of regular sugar, making stevia use quite small with the same sweetness (Sharma et al. 2015). Therefore, stevia can significantly suppress the rate of forest land changes to produce sweeteners.

The development of the world's food and pharmaceutical industries has also made stevia demanded by many national pharmaceuticals and food companies. With its high purity, steviol glycoside is used as a sweetener in foods and beverages in more than 150 countries worldwide (Samuel et al. 2018). Stevia plants are also a commercial commodity grown in Argentina, Brazil, Columbia, Paraguay, China, Japan, Malaysia, South Korea, Vietnam, Israel, Australia, Kenya, the United States, India, China, Canadian, and the Russian Federation (Ciriminna et al. 2018). The stevia sweetener contains zero calories; this makes stevia safe for people with metabolic disorders such as type II diabetes (Clemente et al. 2021). In addition, stevia also contains a complex mixture of triterpenoids, sterols, essential oils, phenols, and flavonoids with functional properties that enhance health (Lemus-Mondaca 2012; Chandra 2015; Clemente et al. 2021).

Stevia cultivation in West Bandung has been carried out by several farmer groups in Lembang. This plantation is done on the land around the forest of Perum Perhutani at an altitude of 700-1,400 meters below sea level (masl). This stevia sweetener production has been marketed, potentially becoming a major commodity in the next few years. The stevia commonly planted is a relatively rapidly harvesting variety of the Sweet Cabbage 3, or stevia CM-3.

Agroforestry allows stevia to obtain lower light intensities from monoculture planting because stevia is planted under the shade of the tree canopy. The low light intensity makes stevia stuck and triggers the production of secondary metabolites in plants (Zhang et al. 2021), so agroforestry patterns are expected to increase steviol glycoside content; farmers in Lembang district harvest stevia with the fastest time in 40 days after planting. Therefore, research on the productivity of stevia at the fastest time (40 days after planting) needs to be done to get the best quality of stevia in the agroforestry system. This study aims to analyze the growth and productivity of agroforestry and monoculture stevia with a harvest time of 40 days after planting.

## MATERIALS AND METHODS

### Study area

The research was carried out from May to August 2022. The research location was at 6°47'14.42"S and 107°39'58.00"E with an altitude of 1,200 m asl. The stevia sampling field is under Cikareumbi Forest coverage, West Bandung District, West Java, Indonesia.

### Procedures

*Stevia cultivation*

Land treatment was done by planting and irrigation using cranes to make the soil clean of weeds and more fertile. Then, we made a straw with a width of 1 m and a length of 3 m. The soil was mixed with chicken manure compost and vegetable residues of 25 kg for the whole straw. After fertilizing the soil, the land was inhabited for a week to be ready for planting. Composite fertilizer is intended to increase soil organic material (BOT) contents.

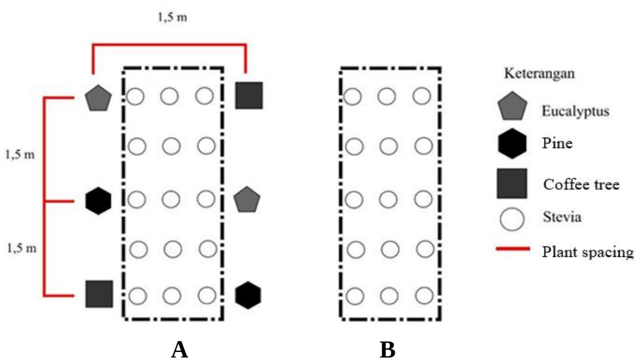


**Figure 1**. Experimental design of: A. Agroforestry, B. Monoculture

The research used was a complete random plan, and the factorial is a planting pattern on 5 sites with two dimensions: monoculture and agroforestry. Stevia grown in agroforestry was under the pine canopy, coffee, and eucalyptus with an average growing distance of 1.5 m, while the monoculture planting is carried out in the same area as agroforestry. However, in monoculture plantings there is only stevia without any shading trees. Each planting block contains 15 stevia plants (Figure 1).

Environmental conditions were measured during stevia planting, namely light intensity and soil conditions. The official World Weather Information website Service obtained average temperature and humidity data. Light intensity was measured once a week at each of the three monoculture and agroforestry planting points with 3 replications. Soil condition analysis was conducted on soil composite samples from the 5 monoculture and agroforestry land points. The pH value of the soil was calculated by the potentiometric method and the C-organic examination was carried out by gravimetric method. N-total was tested by the Kjeldahl method, and the available $P_2O_5$ was tested by the Bray I method, using 0.03 N $NH_4F$ extract and 0.025 N HCl solution. The $NH_4F$ in the Bray extractor formed a chain compound with Fe and Al, which liberate $PO_4^{3-}$ ions. Exchangeable cations were determined by leaching ammonium acetate IN at pH 7.0. $K^+$ exchanged the acidity of $Al^{3+}$ and $H^+$ from 1 M KCl extractor. $Al^{3+}$ and $H^+$ in solution could be titrated with standard NaOH solution to produce $Al(OH)_3$ precipitate and water.

After 40 days of planting, stevia was harvested by uprooting and then separating the leaves; several were prepared in microtubes containing Tris HCl and acetone to be tested for chlorophyll. After being separated, the stevia leaves' wet weight was put into an envelope to be baked. The leaf part was selected because it is a good and marketable product. Stevia was dried in an oven at 40°C for 12 hours or until it was evenly dried and ready for sale. Oven-dried stevia leaves were then ground into powder as an ingredient for the stevioside content test. Stevioside is a compound extracted from stevia leaves. It is a sweetener that stands out among the natural sweeteners. This extract is a white powder composed of stevioside and its anomers, the rebaudioside, responsible for the compound's sweetness (Parpinello et al. 2001).

*Determination of chlorophyll*

The chlorophyll measurement content refers to the method used by Quinet et al. (2012). Samples were taken from plant leaf tips around 08.00-09.00 am, and the sun-facing leaves were selected. Each treatment was taken as many as 3 replications, cut, and weighed to 0.01 g. The sample was stored in a microtube containing an extraction solution of acetone and tris HCl, with a ratio of 85%:15%. The samples were ground with grinding sand in a mortar filled with liquid nitrogen to a small size; after the liquid nitrogen boiled, the samples were ground to a fine powder. The refined sample was put into a measuring cup. The mortar and pestle used for grinding were rinsed with 1 mL of extraction solution three times to dissolve the remaining sample. The extract was transferred into a centrifugation tube and centrifuged for 3 minutes at 10,000 rpm. The filtrate obtained by centrifugation was taken as much as 1 mL and diluted with 3 mL of extraction solution. The absorbance was read using a UV-Vis spectrophotometer at 663 nm for chlorophyll a and 647 nm for chlorophyll b. The absorbance read at the spectrophotometer wavelength was entered into the formula Lichenthaler (1987).

*Stevioside content determination*

Steviol glycoside levels were determined using the Liquid Chromatography Mass Spectrometry (LC-MS/MS) method following Shah et al. (2012). The procedure began with preparing a standard series in 2 mL vials of at least 6 points. Then, 1 g of the liquid test portion or 1 mL pipette of the reconstituted product was put into a 10 mL volumetric flask. Furthermore, the sample was treated with solvent; the solution was then vortexed until homogeneous, and if necessary, the solution was transferred into a 2 mL microtube and then centrifuged in 10.000 rpm. Then the solution was filtered into a 2 mL microtube and 0.1 mL pipette into the vial. Then, 0.9 mL of solvent was added and homogenized. After the solution was homogeneous, the solution was injected into the LC-MS/MS system.

*Instrument conditions*

The instrument used was Water Acquity UPLC I Class + AB Sciex 4500 QTRAP. The LC method used an amide column with a mobile phase of 0.1% ammonia in 80% acetonitrile, a flow rate of 0.3 mL/minute, and a 15-minute isocratic pump system. Furthermore, the MS/MS method used the ESI (-) polarity. The MRM parameters for testing stevioside levels were 803.5 m/z for quadrupole 1 and 641.3 m/z for quadrupole 3. The results were positive if the analyte ion product peak was detected and the retention time for the ion product peak in the sample was similar to the standard retention time with a tolerance limit of ±2.50%.

**Data analysis**

All quantitative data were analyzed using Microsoft Excell and Regression using IBM SPSS 25.

## RESULTS AND DISCUSSION

**Environmental condition**

Table 1 shows that the average daily rainfall in Lembang exceeds the optimal rainfall for stevia growth. The average annual rainfall of Bandung and its surrounding areas, including Lembang district in West Bandung, is 2,206.93 mm, based on data from the last three years, from 2019 to 2021 (World Meteorological Information 2022). The ideal temperature for stevia growth ranges between 6-43°C and an average of 23°C, with rainfall ranging from 1,500-1,800 mm per year (Gunasena et al. 2021). Although both planting patterns received the same rainfall, both conditions received different amounts of water; when it rains, monoculture land is immediately exposed to rainwater, while rainwater on agroforestry lands is blocked by the tree canopy first before falling onto the stevia plants underneath. Moreover, the water absorption in agroforestry is done by stevia roots and tree roots.

The planting location is done in a nearby area, both in monoculture and agroforestry land They are distinguished based on their light intensity Monoculture land is located in an area not covered by pine, eucalyptus, or coffee canopies Measurement results demonstrate this light shortage that the agroforestry planting pattern has less light intensity at 46% than monocultures (Figure 2).

**Stevia growth and productivity**

Stevia grown in agroforestry and monoculture land did not significantly differ in growth development. However, the average of agroforestry stevia was slightly higher than those grown in monoculture (Figure 3). This is because stevia plants in agroforestry experience etiolation; the plant grows faster in height but becomes thin and does not experience leaf development (Armarego-Mariott et al. 2019). Etiolation occurs due to the accumulation of the hormone auxin in the apical part, which is not degraded by sunlight. In low light conditions, auxin becomes active, and the growth of the plant stem becomes taller because meristem cell division is more active, but consequently, the plant becomes weak and only has a few leaves (Hasbullah 2022). This condition occurs because the light intensity received by stevia agroforestry is lower than stevia monoculture (Figure 2).

**Table 1.** Daily temperature, humidity, and rainfall of the study site during stevia planting

| Month | Daily average temperature (°C) | Daily average humidity (%) | Daily average rainfall (mm) |
|---|---|---|---|
| May | 23.96 | 80.74 | 5.88 |
| June | 22.88 | 82.20 | 7.25 |
| July | 23.05 | 80.21 | 4.84 |


**Figure 2.** Light intensity in monoculture and agroforestry stevia planting areas

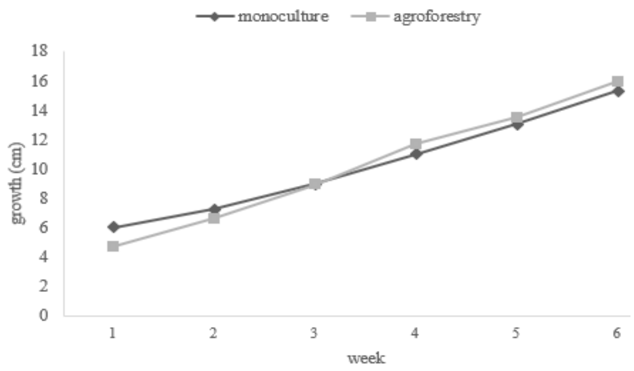


**Figure 3.** Graph of stevia high growth in agroforestry and monoculture with a harvest time of 40 days after planting (the data is taken from the average of 15 stevia samples)

The stevia leaf portion is very important for its commercial products because most stevia processed products are taken from the leaf parts. Figures 4.A and 4.B show that stevia has the largest number of leaves, and the largest leaf area is on the monoculture plant. The average number of leaves is also related to light intensity, where stevia planted in agroforestry experiences stunted leaf development. Meanwhile, leaf size is an important parameter in the growth and productivity of plants. Leaves with large surfaces allow plants to do more photosynthesis. These different leaf sizes cause differences in the production of stevia biomass. The larger the leaf area, the greater the plant's capacity for photosynthesis, which can increase the total plant dry weight. Based on the number of leaves and leaf area produced, in terms of yield quantity, monoculture stevia is superior compared to agroforestry stevia.

Figures 5.A and 5.B show the wet weight of leaves in monocultures is higher than in agroforestry; sufficient light intensity also affects the wet weight (Amanullah 2015). The productivity of stevia is determined by the ratio between dry weight and wet weight produced. This is because this dry weight is what is sold in the market as a rough product of stevia. Generally, both cultivation patterns have the same average rainfall and are sufficient for stevia growth (Table 1).

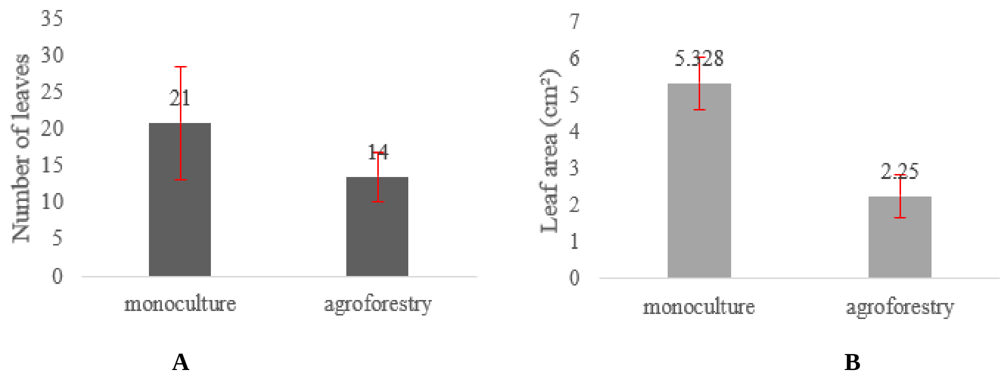


**Figure 4.** Graph of: A. The average number of leaves; B. The average leaf area of stevia harvested 40 days after planting. Different letters indicate significantly different values in the ANOVA test

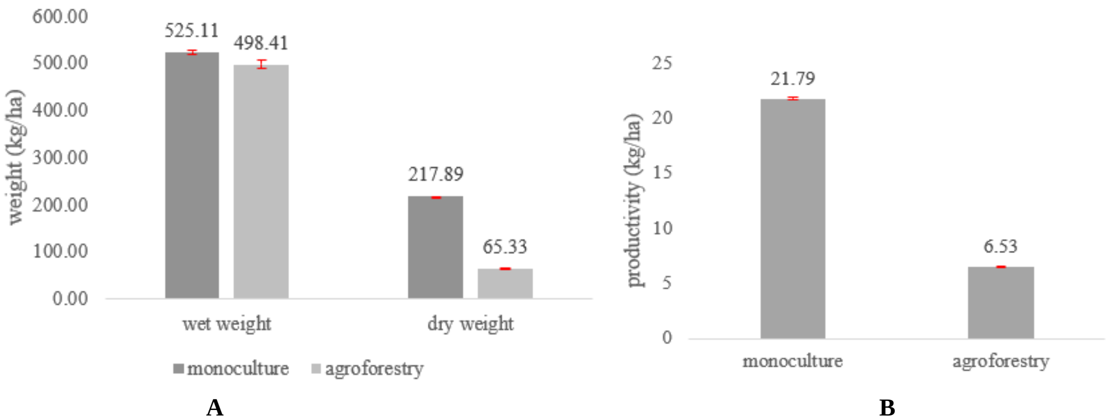


**Figure 5.** Graph of: A. Weight; B. Productivity stevia harvested 40 days after planting

The dry weight value of stevia in monoculture is higher than in agroforestry. The low weight of agroforestry stevia occurs due to shade, which causes the leaves to become thinner (Sutuliene 2022). This condition aligns with research by Husna et al. (2018), which states that stevia under shade has a lower weight than stevia in monoculture. This dry weight is influenced by fresh weight and the plant's water content reduction. Stevia in monoculture with more water than agroforestry experienced less weight loss (59%) than agroforestry (87%) (Table 2). This calculation is obtained from the comparison between the difference between wet weight and dry weight with wet weight.

$$\text{Weight loss} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100\%$$

Based on the results of the calculations of the dry weight obtained from each planting bed with an area of 0.0003 ha, it was found that the estimated productivity per hectare from monoculture land was 21.79 kg/ha, and from agroforestry land was 6.53 kg/ha.

## Physico-chemical assesment of the stevia samples

Test chlorophyll showed that the total content of chlorophyll a and b belongs to stevia crops grown in agroforestry that is higher than in monoculture (Figure 6). In low-light conditions, plants multiply the amount of chlorophyll to maximize light capture. In line with research by Dai et al. (2009), plants grown under shading are better at optimizing light absorption efficiency by increasing pigment density per unit of leaf area. Other studies also state that the chlorophyll content will increase with increasing shade (less light available) (Khalid et al. 2019), the chlorophyll b/a ratio is greater, the leaves are thinner, the palisade cells are shorter, and the rubisco concentration is less (Choi et al. 2021). Several factors also influence chlorophyll content, they are climate (Li et al. 2018) and chemical process in the leaf (Pareek et al. 2017).

The stevioside content of stevia plants in agroforestry lands (250.39 mg/kg) has higher values than in monoculture (100.73 mg/kg) (Figure 7). The Pearson correlation shows that there is a relationship between the amount of chlorophyll and the stevioside content in stevia plants (Figure 7). The ANOVA tests on the chlorophyll and stevioside parameters show that the significant value is less than 0.05 (Table 3), which means a correlation between chlorophyll and the amount of stevioside with the estimating equation Y=2.237+0.003X (Figure 8). Estimating stevioside content using chlorophyll allows farmers to measure the quality of stevioside using it. Moreover, the cost of testing chlorophyll content in the laboratory is cheaper than testing stevioside levels.

Chlorophyll is a pigment found in chloroplasts. The greater the amount of chlorophyll, the greater the number of chloroplasts. Biosynthesis of steviol glycosides occurs in the leaves, and these products are transported to different parts of the plant. Chloroplasts in the leaves play an important role in the biosynthesis of the steviol glycoside and act as precursors. This condition is also related to the presence of shade and light intensity. The shading in agroforestry affected the low light intensity received by stevia; this low light intensity increasing the amount of chlorophyll in the leaves. The greater the amount of chlorophyll, the more stevioside levels increase.

**Table 2.** Stevia leaves loss weight from monoculture and agroforestry

| | Wet weight (kg/ha) | Dry weight (kg/ha) | Weight loss (%) |
|---|---|---|---|
| Monoculture | 525.11±1.27 | 217.89±0.04 | 59 |
| Agroforestry | 498.41±2.50 | 85.33±0.01 | 87 |

**Table 3.** Results of variance analysis of the correlation between chlorophyll and stevioside

| | Unstandardized coefficient | | Standardized coefficients | t | Significant Value |
|---|---|---|---|---|---|
| | B | SE | Beta | | |
| Stevioside | 0.003 | 0.001 | 0.930 | 5.078 | 0.007 |
| (constant) | 2.237 | 0.131 | | 17.049 | 0.000 |

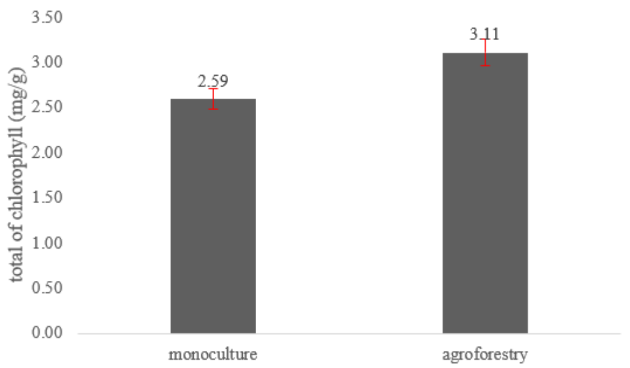


**Figure 6.** Graph of chlorophyll in stevia harvested 40 days after planting in monoculture and agroforestry

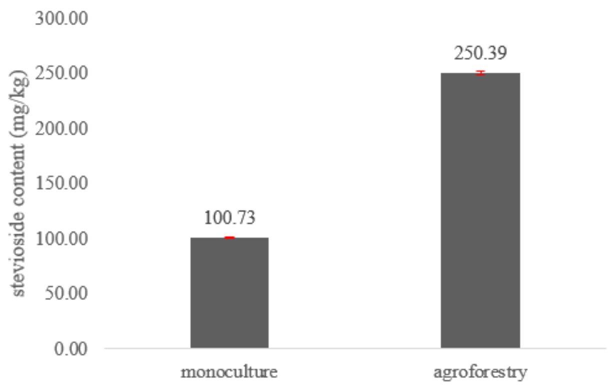


**Figure 7.** Graph of stevioside content in stevia plants harvested 40 days after planting from monoculture and agroforestry. The resulting value comes from 1 g of tested stevia solution. The test solution dissolved 1 g stevia leaf powder with 50 mL of distilled water

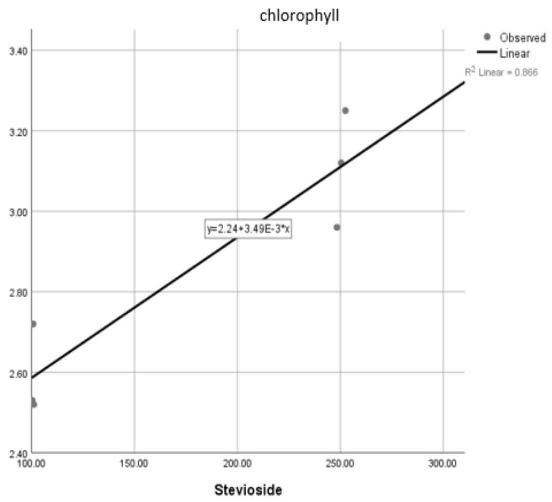


**Figure 8.** Graph of Pearson correlation test between chlorophyll and stevioside content in stevia

In conclusion, stevia in agroforestry had higher chlorophyll and stevioside content than monoculture, although not as good as stevia in monoculture, which had higher height, number of leaves, leaf area, wet weight, dry weight, and productivity compared to agroforestry.

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