

The Effect of Apical Pruning and Eco Enzyme Application on Optimizing Growth and Sugar Accumulation in *Stevia rebaudiana*

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Abstract

Stevia is a medicinal plant that contains stevioside, rebaudioside A and steviol with a sweetness level that is 300 times sweeter than sucrose. High temperatures can stimulate earlier flowering and will reduce the nutritional content. Apical pruning has the potential to stimulate the growth of lateral shoots, thereby increasing plant biomass. At the same time, eco enzyme, as an organic material resulting from the fermentation of kitchen waste, can be a biocatalyst in accelerating chemical reactions, which ultimately supports the growth and production of secondary metabolites. However, the effectiveness of these two treatments in cultivating *Stevia* has not yet been studied in depth, especially in the context of increasing natural sugar accumulation. This research aims to determine the effect of pruning and *the eco enzyme* on the production and accumulation of sugar in *stevia*. The study was conducted in the lowlands and used a factorial Randomized Block Design. Pruning (4 and 8 WAP) was used as the first factor and Eco Enzyme (0, 1, 2, and 3 ml/l water) was used as the second factor. The research result showed that application of 4 WAP shoot pruning and 1 ml/l water eco enzyme could increase shoot dry weight, fresh weight of leaves, leaf epidermis, number of stomata, and total sugar accumulation in *stevia*.

1. Introduction

Stevia rebaudiana Bertoni, popularly known as ‘candy leaf’, is a sweet native herb of Paraguay. It became economically important for its significant contribution to the sugar and beverage industry throughout the world (Das, A. et al., 2018). Steviol glycosides, found in *Stevia rebaudiana*, exhibit superior sweetening proficiency to sucrose and are noncaloric, noncariogenic and nonfermentative (Orellana-Paucar, A., 2023). *Stevia*’s sweetness is due to steviol glycosides, which are 250–300 times sweeter than sucrose and has been suggested to have health benefits such as anti-hypertensive, anti-obesity, anti-diabetic, antioxidant, anti-cancer, anti-inflammatory, and antimicrobial effects (Rybchuk, L, et al., 2021). The global *stevia* market is experiencing rapid growth, projected to reach \$1.1 billion by 2026, reflecting strong consumer and industry interest in healthier sugar alternatives. The *Stevia* market is estimated to be USD 1.14 billion in 2028 due to its acceptance in the food and beverage industry (Rivera-Avilez, J. et al., 2021).

Stevia contains a diverse array of steviol glycosides, which are responsible for its intense sweetness and are widely used as natural, low-calorie sweeteners. The main steviol glycosides identified in *stevia* leaves include stevioside, rebaudiosides (A, B, C, D, E, F, and M), steviolbioside, isosteviol, rubusoside, and dulcoside A, with stevioside and rebaudioside A being the most abundant and well-studied for their sweetening properties and safety profile (Islam, M, et al., 2025). *Stevia rebaudiana* leaf extracts are recognized for their strong antioxidant properties, largely attributed to their high content of polyphenols and flavonoids. Studies comparing the antioxidant activity of *stevia* leaf extracts to ascorbic acid (vitamin C) show that while *stevia* extracts have higher total phenolic content, their direct free radical scavenging ability (measured by IC₅₀ in DPPH assays) is generally lower than that of ascorbic acid

meaning higher concentrations of stevia extract are needed to achieve the same antioxidant effect as ascorbic acid (Kushwaha, S. et al., 2024).

Stevia cultivation in lowlands is rarely done due to the need for special handling in order to adjust to environmental differences and nutrient supply. Stevia is a shrub that grows at an altitude of 500–1000 meters above sea level with a fairly cool temperature of 14–27°C and rainfall between 1600–1850 mm/year with 2–3 dry months (Putnik, P. et al., 2020). Stevia is a short-day plant with a critical period of 12–13 hours and has a tendency for a shorter vegetative stage and earlier flowering when under a photoperiod of 12 hours (Ceunen, S. et al., 2011). With optimal soil and nutrient management, lowland stevia can achieve dry leaf yields of up to 2.7 tons/ha and stevioside content up to 6.2%, which can surpass highland yields under standard fertilization. However, this plant is in dire need of water availability, because the stems and leaves will easily wither if they do not get enough water. Sufficient water availability is a limiting factor for stevia to be able to grow and reproduce high (Lemus, M. et al., 2012).

A major challenge in stevia cultivation is its tendency to flower rapidly, which significantly reduces the stevioside content in the leaves often by as much as 50% after flowering. Research consistently shows that stevioside and other valuable steviol glycosides reach their highest concentrations during the late vegetative or pre-flowering stages, and decline sharply once the plant begins to flower (Shaari, K. et al., 2021). Pruning, specifically shoot pruning or decapitation, is an effective agronomic practice to enhance stevia productivity by promoting lateral shoot growth and increasing the number of branches and leaves. Research demonstrates that pruning breaks apical dominance, encouraging dormant axillary buds to develop into new branches, which leads to a significant rise in leaf biomass the primary source of steviol glycosides (Osman, M. et al., 2011). Pruning can increase dry leaf production of stevia by 1.36 tons/ha (2010) and 1.59 tons/ha (2011), or by 13 and 17%, respectively, compared to unpruned plants (Pala, P. et al., 2013).

Applying eco enzyme as a liquid organic fertilizer after pruning can significantly boost stevia plant productivity. Eco enzyme acts as a biocatalyst, enhancing plant metabolism and stimulating growth, which leads to faster and more robust development of new shoots and leaves. Eco Enzyme is a complex organic solution made from fermented waste such as fruit or vegetable pulp, molasses, and water in a ratio of 1:3:10. The enzymes contained in eco enzyme are biological catalysts that can accelerate chemical reactions that break down complex molecules from organic materials into simpler molecules (Supriyatna, A. et al., 2015). Eco enzyme can be used as a biostimulant because it contains nitrates (NO_3) and growth factors such as α -amylase, protease, and lipase enzymes, which can act as stimulants for plant cell growth and development (Arun, C. et al., 2016). In addition, eco enzymes also contain organic acids such as acetic acid and several plant nutrients such as N, P, and K, as well as lactic acid bacteria that play a role in plant growth (Susilowati, L.E. et al., 2021). Studies have shown that stevia plants treated with eco enzyme exhibit increased plant height, larger and more numerous leaves, and greater fresh weight at harvest compared to those not receiving eco enzyme (Shaarim K. et al., 2021). This improved growth translates into higher yields and greater income for farmers, as demonstrated by

research showing that stevia farming with eco enzyme application resulted in positive income gains, while farms without it experienced losses (Momongan, R. et al., 2023). Based on the above statement, the author would like to conduct research regarding adaptaion strategy of *Stevia rebaudiana* in the lowlands through pruning and eco enzyme based on morphology characteristics.

2. Methodology

2.1. Study Area

This research was conducted on research land located at 03° 53′ 09.7″ North Latitude, 098° 64′ 00.0″ East Longitude with an altitude of ± 32 meters above sea level. The analysis was carried out at the Biotechnology and Pests Diseases Laboratory, Faculty of Agriculture, University of North Sumatra, at Jl. Dr. A. Sofian No.3, Padang Bulan, Kec. Medan Baru, Medan. This research was conducted from June to August 2024. The plant material used in this study was *Stevia rebaudiana* Bertoni obtained from local farmers in Kediri, East Java, Indonesia. The plants were cultivated under conventional agricultural practices in privately managed farmland. Healthy plants with relatively uniform growth and morphological characteristics were selected and used as experimental materials for the apical pruning and eco-enzyme treatments.

2.2. Procedures

The design used in this research was a factorial complete randomized block design with 2 factors and 3 replications. The first factor is pruning (4 and 8 WAP), while the second factor is Eco Enzyme (0, 1,2, and 3 ml/l water). Pruning is carried out in accordance with the research treatment, namely 4 WAP and 8 WAP on the fifth leaf sitting from the tip of the shoot., while Eco enzyme Eco enzyme is applied once every 2 weeks with a concentration determined according to the treatment, namely 0, 1, 2 and 3 ml/l water. Eco enzyme is given by spraying it on the entire plant canopy. To determine the volume of eco enzyme spray that will be used, calibrate first with a 1 liter handsprayer. Control of nuisance organisms is focused on minimizing pest attacks and prioritizing plant-based control. The pest many attacks include aphids (*Aphis* sp), armyworms (*Heliothis* sp.), grasshoppers and caterpillars/span caterpillars. This type of pest can be controlled by biological pesticide neem seed extract (Manginsela, E. et al., 2023).

Stevia harvesting is carried out when the plants are 75 days old. The ideal time for harvesting is in the morning. Harvesting is done by cutting the stems about 10–15 cm from the ground using pruning shears. Post-harvest activities for stevia include picking the leaves attached to the stems and sorting them to separate the low-quality leaves. Next, washing is carried out to clean the leaves of dirt to maintain the quality of the stevia (Y. M. Killa. *et al.*, 2023). For drying stevia leaves, use the air drying method in a dry, shaded place, which takes about one week. The dried leaves are yellowish-green in color with a maximum moisture content of 10%, a minimum stevioside content of 10%, and a maximum impurity content of 3% (Rukmana., 2003).

Table 1
Variables and Parameters

Variables	Description	Parameters
Agronomic Parameters	Dry Weight of Leaves	Dry weight of plants at harvest, by weighing the entire plant using an electric scale. Samples were separated into leaves, twigs, and stems, then weighed separately. Fresh leaves were oven-dried for 2x24 hours at 70°C. Dry samples were weighed, then the moisture content and dry weight of all leaves were calculated.
	Fresh Weight of Leaves	Leaf wet weight was observed by cutting the crown of the plant, then the wet weight of the leaves was measured by weighing the plants. The wet weight of the leaves was measured at the end of the study using digital scales.
Anatomical Parameters	Leaf Epidermis	Observation of leaf epidermal parameters was conducted when the plants were 10 week after planting using microscopy techniques after proper sample preparation. For the leaf epidermis, a 1–2 cm section of the leaf was cut and immersed in a staining solution such as safranin to clarify the epidermal structure. Epidermis prints were made by attaching clear tape to the underside of the leaf and then attaching it to a microscope slide. The preparation was then observed using a light microscope with appropriate magnification to identify and analyze the epidermal structure of the leaf.
	Number of Stomata	The calculation of the number of stomata is carried out through several stages involving microscopy techniques. The number of stomata is counted by selecting at least five random fields of view on each specimen. The number of stomata in each field of view is counted and recorded, then expressed as the number of stomata per unit area, for example, stomata per mm ² .
Biochemical Parameters	Total Sugar Content	Analysis of total sugar content of stevia leaves was determined using the phenol sulfuric acid method with absorbance measured at 630 nm using a spectrophotometer (Mishra, P.K. <i>et al.</i> , 2010). For the extraction step, 50 mg of sample was placed in a 15 ml centrifuge tube and 5 ml of 80% ethanol was added in a water bath at 80°C for 30 minutes. Filter and place the extract into a 50 ml measuring flask, leaving the residue in the centrifuge tube. Repeat this extraction 3 more times. The supernatant in the 50 ml measuring flask is then filled with distilled water. This extract is used for total soluble sugar content. For analysis, 0.5 ml of sugar extract is mixed with 4.5 ml of 80% ethanol in a test tube. Add 10 ml of anthrone reagent (100 mg anthrone, 50 ml H ₂ SO ₄ 95%). The mixture is incubated in boiling water for 7.5 minutes. The reaction is stopped in an ice bath.

3. Result

3.1 Fresh and Dry Weight of Leaves

The analysis of fresh and dry stevia leaf biomass revealed that the interaction between shoot pruning and eco-enzyme application significantly enhanced leaf yield. The combination of shoot pruning at 4 weeks after planting and eco-enzyme application at 1 ml L⁻¹ produced the highest fresh and dry leaf weights, whereas the lowest biomass was observed in plants pruned at 8 weeks after planting with eco-

enzyme application at 3 ml L⁻¹. Plant production is fundamentally determined by both the total amount of dry matter accumulated and how that dry matter is distributed among plant organs, especially those harvested for yield. Dry matter accumulation typically follows a sigmoid (S-shaped) curve, with the rate and final amount influenced by environmental factors such as water and nutrient availability, as well as genetic improvements in crop varieties (Xu, J. et al., 2022).

Description: Number followed by different letters indicate significant according to Duncan's Multiple Range Test at level = 5%

Pruning shoots can effectively stimulate vegetative growth in plants by redirecting energy and resources toward the development of new shoots, which leads to increased canopy wet and dry weights, as observed in crops like tea where pruning enhances shoot number and biomass through hormonal and metabolic changes (L. Ye. *et al.*, 2024). At 4 MST, pruning has better growth potential compared to plants pruned at an older age. These plants are still in the active vegetative phase, which triggers more shoots and leaves to grow, contributing to an increase in wet crown weight. Pruning treatment carried out when entering the generative phase did not affect the total dry weight of the plant (Jeyaraj, A. et al., 2020). The timing of shoot pruning will affect plant growth. When shoot pruning occurs during the vegetative phase, it increases vegetative organs, whereas if shoot pruning occurs at the end of the vegetative phase, it accelerates the formation of generative organs (Yanti, U.D. *et al.*, 2019).

Description: Number followed by different letters indicate significant according to Duncan's Multiple Range Test at level = 5%

The application of eco enzyme, a nutrient-rich solution derived from fermented fruit and vegetable waste, further supports optimal plant growth by increasing the availability of essential nutrients such as nitrogen, phosphorus, and potassium (Saitama, A. et al., 2017). The application of eco enzyme contains various enzymes that can increase biological activity in the soil. Applying eco enzymes will produce nitrogen (N) nutrients that play a role in cell formation, so plants require a large enough amount during the vegetative phase (Refilda, R. et al., 2025). The increase in wet and dry weight of stevia plant crowns is thought to be due to enzymatic activity in eco enzymes. Enzymes such as amylase and protease play a role in breaking down organic matter into simpler compounds that are easily absorbed by plants (Sitawita, W. *et al.*, 2023). This contributes to increased cell formation and cell division, which affects plant weight.

3.2 Number of Stomata

The stomatal density analysis demonstrated that the interaction between shoot pruning and eco-enzyme application significantly influenced stomatal number. The highest stomatal density (16.33) was observed in plants subjected to shoot pruning at 4 weeks after planting combined with eco-enzyme application at 1 ml L⁻¹, whereas the lowest value (13.00) occurred in plants pruned at 8 weeks after planting with the same eco-enzyme concentration. The high number of stomata of stevia which is a C3 plant has an important role as a way of exchanging CO₂, O₂ and H₂O gases during photosynthesis, respiration and

transpiration. A higher number of stomata on plant leaves can accelerate metabolic processes such as photosynthesis by increasing the capacity for carbon dioxide uptake, which is essential for photosynthetic activity. Additionally, more stomata enhance transpiration, the process by which water vapor exits the leaf, which not only supports nutrient uptake but also provides evaporative cooling that helps reduce leaf temperature an important benefit for stevia and other crops grown in hot, lowland environments (Kambale, R. et al., 2023).

High light intensity increases the number of stomata, while low light decreases it. This effect is strongest in the early stages of leaf development. The number of stomata affects photosynthesis, water use efficiency (WUE), and tolerance to environmental stress. High stomatal density accelerates the photosynthetic response to light changes, but can reduce water efficiency. Moreover, Nitrogen can increase the number of stomata, thereby increasing the rate of transpiration and CO₂ absorption for photosynthesis (Nadiyah, S. et al., 2023). The number of stomata is influenced by the number of leaves on a plant; if a plant has many leaves, the photosynthesis process can proceed well. Based on the results of laboratory tests, eco enzyme contains 0.28% nitrogen, which can promote vegetative growth in plants, including the formation of chlorophyll in leaves, thereby increasing the number of stomata in leaves. providing sufficient N nutrients to plants can help increase the width, length, and number of leaves (Aulia, I. et al., 2022).

Description: Number followed by different letters indicate significant according to Duncan's Multiple Range Test at level = 5%

3.3 Leaf Epidermis

The epidermal thickness of stevia leaves was significantly affected by the interaction between shoot pruning and eco-enzyme application. The greatest epidermal thickness (17.24) was observed in plants subjected to shoot pruning at 8 weeks after planting combined with eco-enzyme application at 1 ml L⁻¹, whereas the lowest value (11.24) occurred in plants receiving neither pruning nor eco-enzyme treatment. The increase in cell size or the state of enlarged cells is closely related to the growth of elongated and widened leaves. An increase in leaf cell size is one way plants adapt to high temperature conditions, as larger cells can help maintain internal water status and support efficient physiological processes under heat stress (Qi, D. et al., 2020). Epidermal thickening plays a major function in reducing tissue damage caused by abundant solar radiation and maintaining a high level of photosynthesis. The enzymes contained function as catalysts that can accelerate the reaction rate by 10⁸ to 10¹¹ times faster than when the reaction does not use a catalyst (Marks, D. et al., 2014). The thickness of leaves in each plant varies and can be influenced by the amount of sunlight exposure. Solar radiation affects leaf thickness; the palisade layer will thicken as the amount of sunlight received by the plant increases (Saputri, D. et al., 2019).

The epidermal tissue protects the inner tissues of leaves from excessive water loss (transpiration), pathogen attacks, and damage from UV radiation. The epidermis also regulates gas exchange (CO₂ and O₂) and water evaporation through stomata. Most CO₂ intake and water loss occur through stomata,

while the epidermis and cuticle limit diffusion when the stomata are closed. Thick leaves will have more cells than thin leaves. A high cell count has a higher photosynthetic capacity. Thick leaves result in a high volume to surface area ratio, therefore, for the same tissue volume, the transpiration surface area is lower (Hariandi, D. et al., 2019). High light intensity at low altitudes causes leaves that are directly exposed to sunlight to adapt by becoming thicker, such that the more palisade parenchyma cells there are, the thicker the leaf blade becomes [36]. Thus, mature leaves no longer develop palisade parenchyma, and the coral tissue becomes more abundant, causing the transpiration rate to increase.

Description: Number followed by different letters indicate significant according to Duncan's Multiple Range Test at level = 5%

3.4 Total Sugar

The total sugar content of stevia leaves was influenced by the interaction between shoot pruning and eco-enzyme application; however, the effect was not statistically significant. The highest total sugar content (27.55) was observed in plants subjected to shoot pruning at 8 weeks after planting combined with eco-enzyme application at 3 ml L⁻¹, whereas the lowest value (15.20) occurred in plants pruned at 8 weeks after planting with eco-enzyme application at 1 ml L⁻¹. Research on phalsa plants indicates that total sugar content is not significantly affected by pruning height alone; rather, significant increases in total sugars are observed when pruning is combined with certain chemical treatments, such as foliar application of zinc sulfate (ZnSO₄) (Tripathi, V. *et al.*, 2025). However, pruning itself did not always cause dramatic changes in total sugar content in some crop varieties. Pruning treatment at 4 weeks showed higher total sugar content in lowland stevia plants. This suggests that pruning stimulates growth and accumulation of total sugar content in the plant. Pruning the plants will optimize the process of photosynthesis and carbohydrate accumulation which contributes to the increase in total sugar content. An increase in the rate of photosynthesis contributes directly to the accumulation of total sugar in plants, an increased rate of photosynthesis will produce more glucose and carbohydrates (Effendy, I. et al., 2020).

Summer pruning imposed higher content of sucrose, fructose and sorbitol in flower bud of each canopy section but higher content of glucose and total sugars in flower bud of outer and upper sections. The higher sugar content is probably related to improved light conditions after summer pruning. Better light conditions influence photosynthesis, resulting in higher accumulation of sugars in flower buds and other storage organs (Usenik, V. et al., 2008). Higher sugar content in flower buds could lead to better fertilization and consequently higher yields. Flower buds from the upper canopy section had the highest, while the inner section had the lowest fructose and sorbitol content. Irradiance plays an important role in this case. Low fructose and sorbitol content in flower buds from the inner canopy may be related to low availability of light there (Bartolini, S. et al., 2013). The transport of sugar in plant, its transport direction, and volume are affected by the position of the sink and the relative strength of the sink. Carbohydrates produced in leaf mesophyll are loaded into the phloem systems and unloaded in energy-consuming or storage tissues (sinks) (Roch, L. et al., 2019).

4. Limitations and future prospects

This study was conducted in a single lowland location and within one growing season, which may limit the generalizability of the findings across different agroecological conditions and climatic variations. The analysis focused on agronomic, anatomical, and total sugar parameters, without distinguishing individual steviol glycosides such as stevioside and rebaudioside A that are critical for determining sweetness quality and commercial value. In addition, the range of pruning times and eco enzyme concentrations tested was relatively limited, and variations in eco enzyme composition due to differences in raw materials or fermentation processes were not characterized. Furthermore, the underlying physiological, metabolic, and molecular mechanisms associated with pruning- and eco enzyme-induced responses were not directly examined.

Future studies should evaluate the effects of pruning and eco enzyme application across multiple locations and seasons to enhance the robustness of the results, while incorporating more detailed biochemical analyses of specific steviol glycosides using advanced techniques such as HPLC or LC–MS. Integrating metabolomic and transcriptomic approaches would provide deeper insights into the regulatory pathways governing carbohydrate metabolism and secondary metabolite biosynthesis in stevia. Additionally, assessing the long-term agronomic, ecological, and economic impacts of eco enzyme use would support the development of sustainable and optimized cultivation strategies for stevia in lowland environments.

5. Conclusions

The interaction between 4 MST shoot pruning and the application of eco enzyme 1 ml/l water can increase the wet and dry weight of leaves, the number of stomata, and total sugar accumulation of *Stevia*. While the thickness of the leaf epidermis tends to be thicker due to the treatment of pruning, 8 MST and eco enzyme 1 ml/water. Through this research, farmers and scientists can find out the best pruning time and eco enzyme concentration to maximize stevia growth and sugar accumulation in stevia.

Declarations

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Authors' Contributions

Riska Wahyuni designed the research concept, conducted the literature search, data extraction, and analysis, and drafted the initial manuscript. Chairani Hanum assisted in data analysis and interpretation.

Lisa Mawarni contributed to literature review and manuscript editing.

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Data Availability Statement

No datasets were generated or analysed during the current study.

Ethical approval for Plant Use

The collection and use of *Stevia rebaudiana* in this study complied with institutional, national, and international guidelines for plant research.

Permission for Plant Collection

The plant materials used in this study were obtained from privately cultivated farmland in Kediri, East Java, Indonesia, with the permission of the farmers. Therefore, no specific permits or licences were required for the collection of plant materials.

Consent for publish

Not applicable.

Competing Interest

The authors declare that there no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication or falsification, double publication or submission, and redundancy, were observed by the author

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Figures

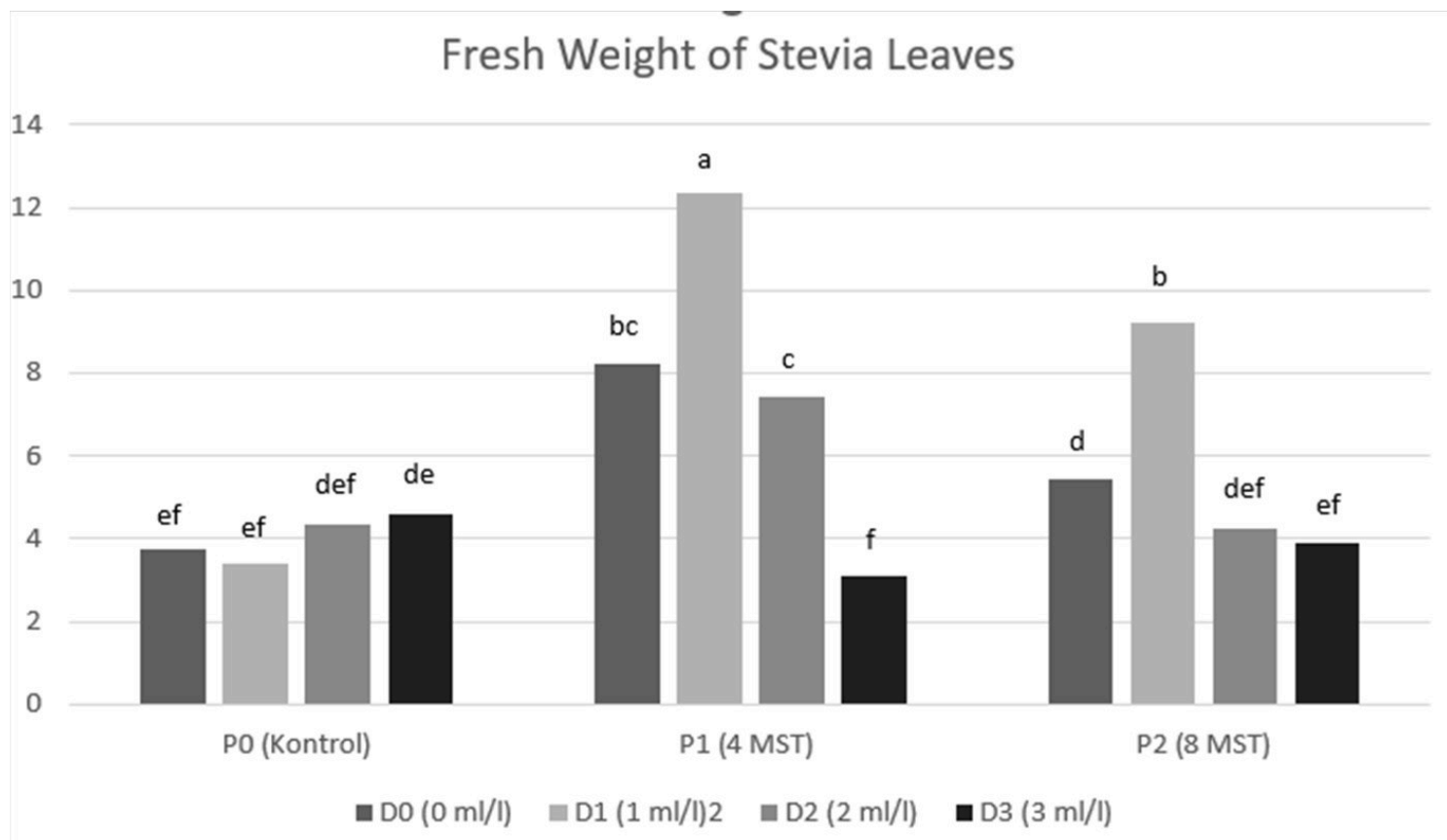


Figure 1

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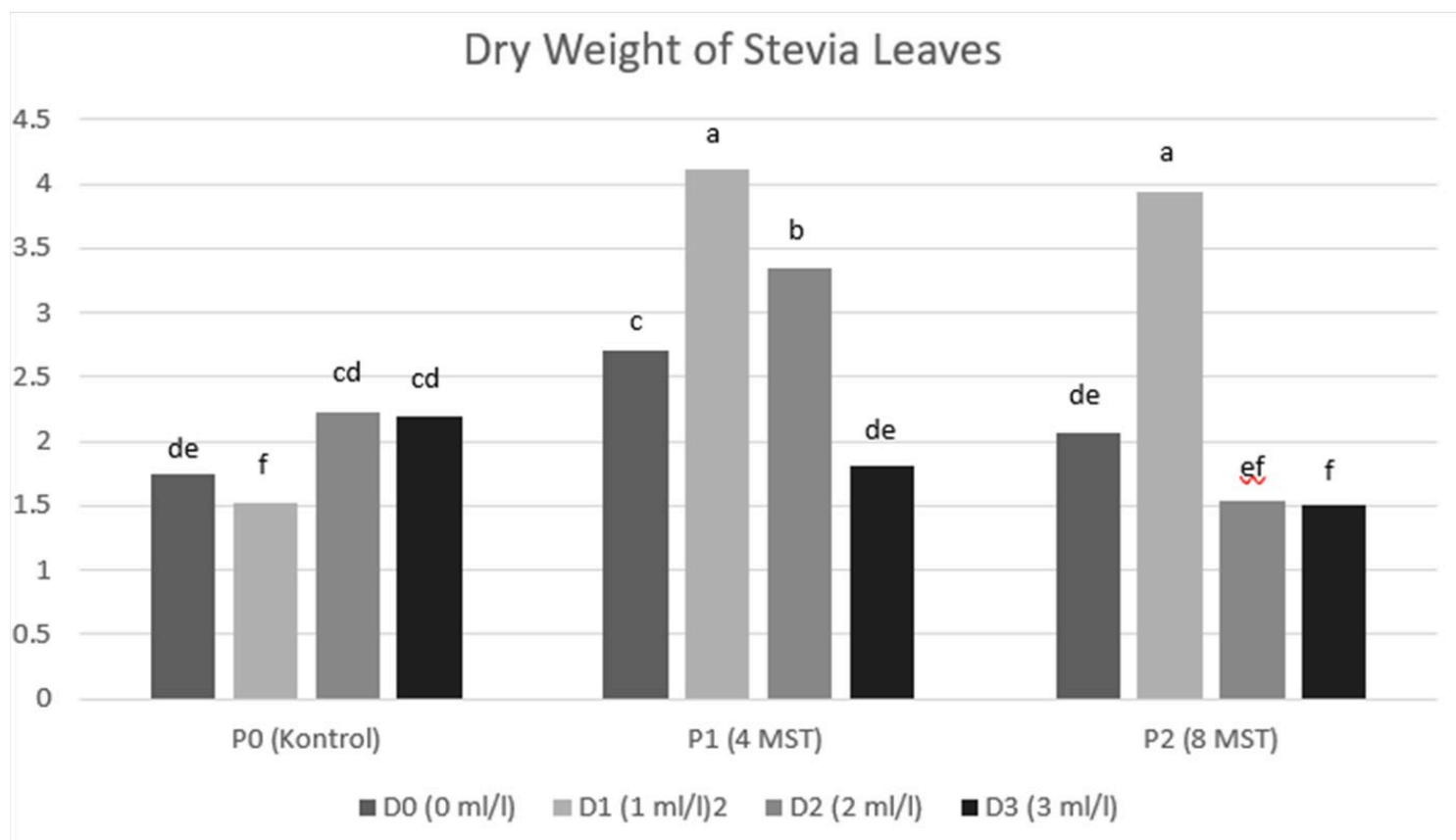


Figure 2

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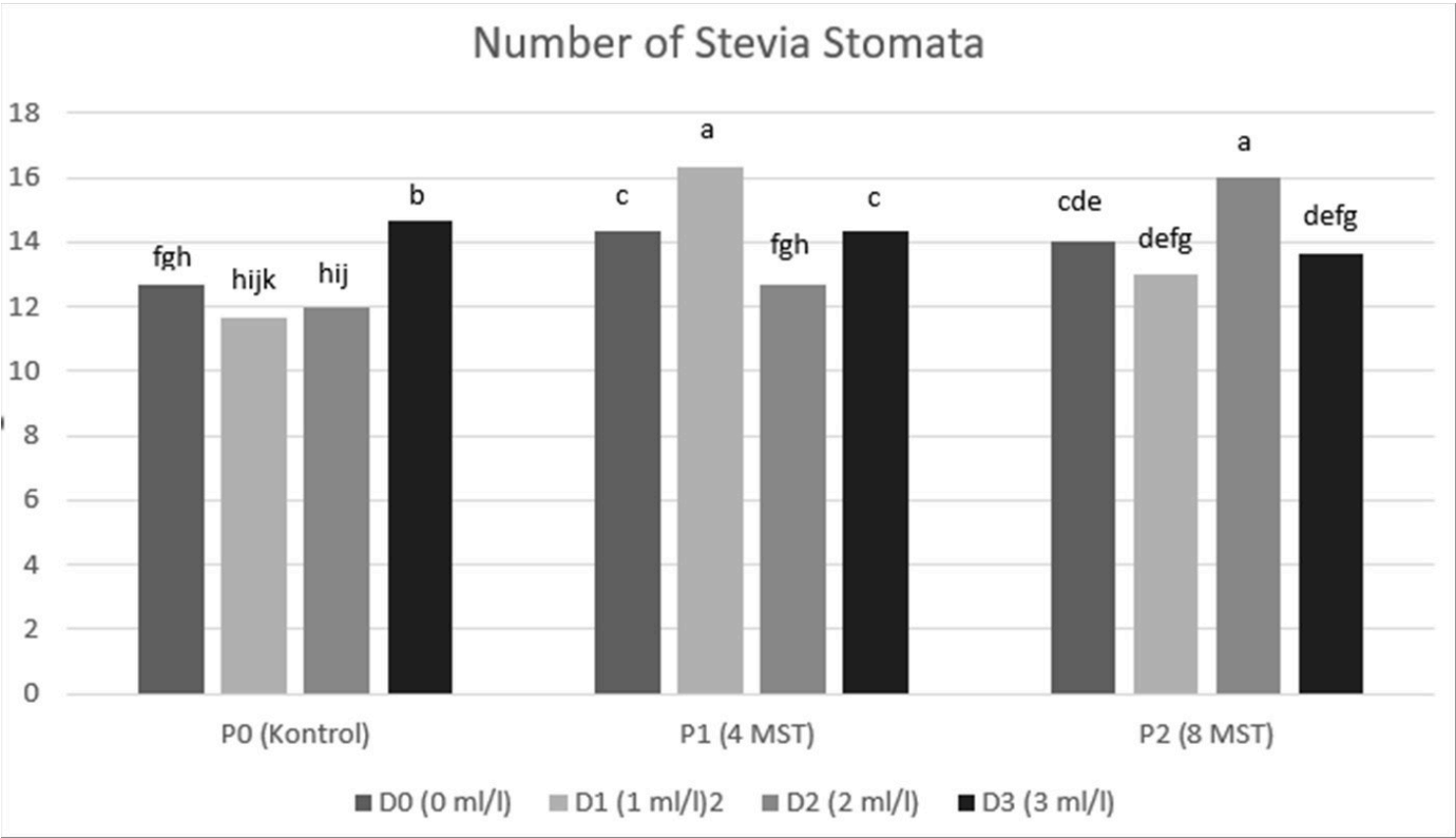


Figure 3

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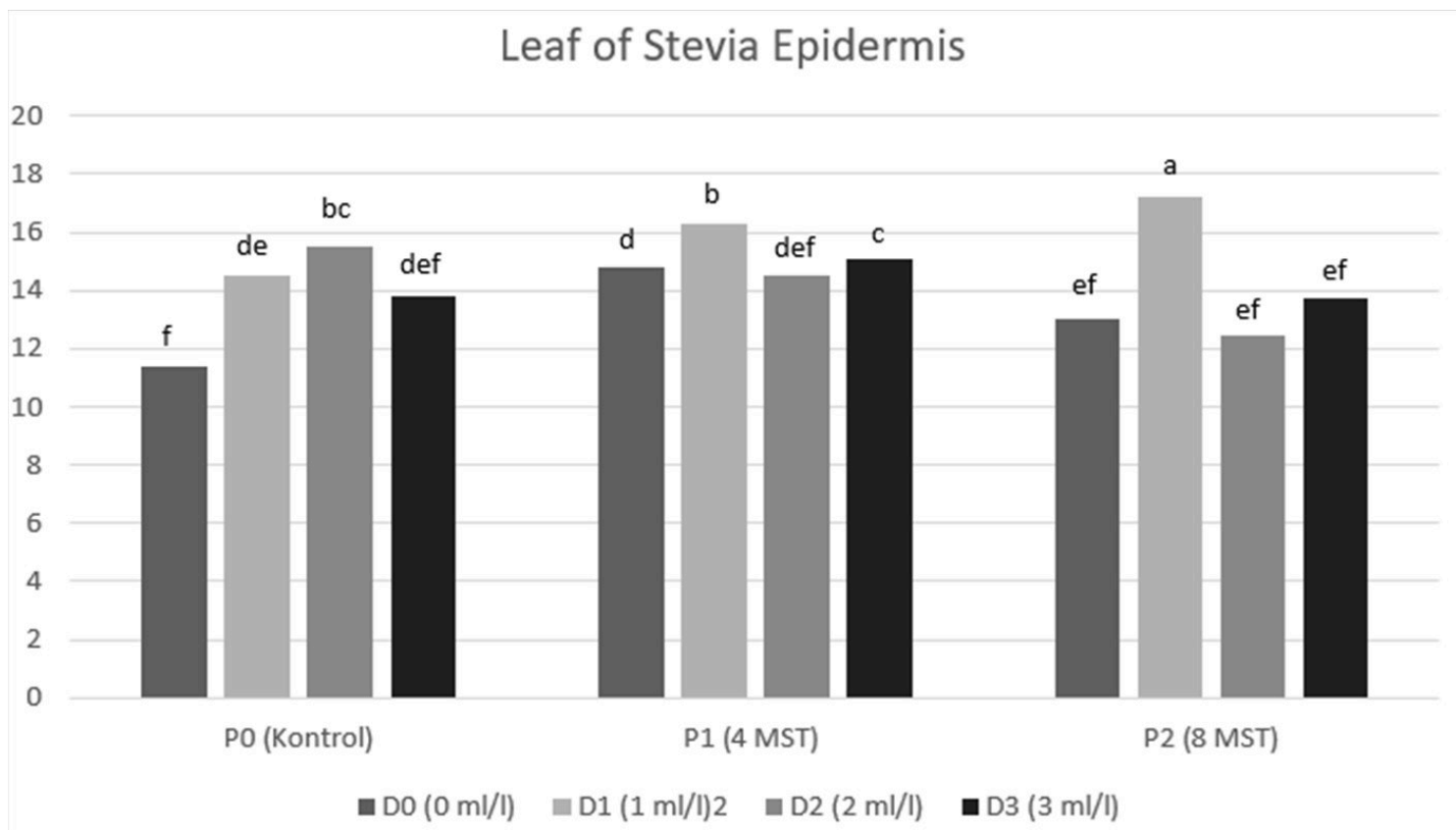


Figure 4

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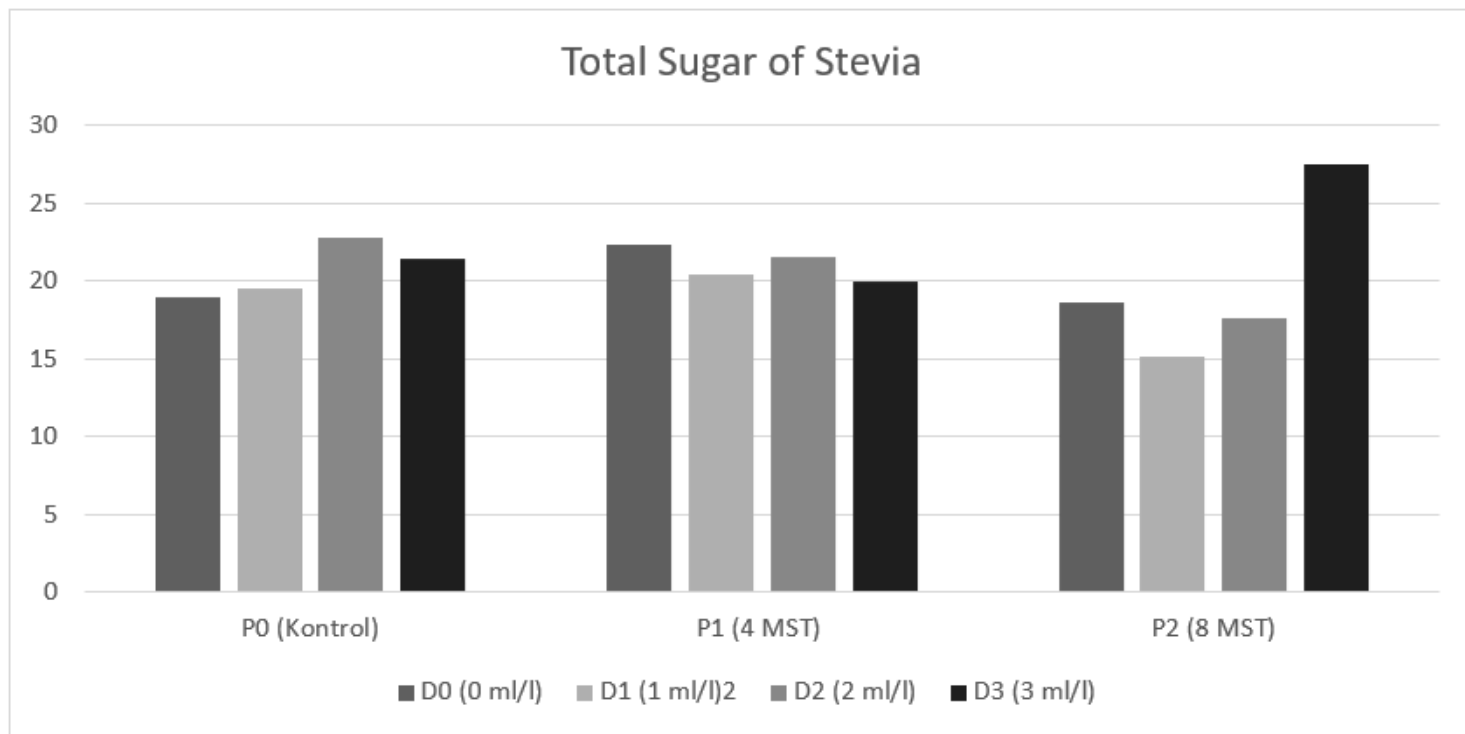


Figure 5

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