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Optimum Fertilization Technology to Improve Crop Productivity of Stevia (*Stevia rebaudiana* Bertoni)

Mohammad Cholid, Sesanti Basuki, Budi Hariyono, Fitriningdyah Tri Kadarwati and Prima Diarini Riajaya

Research Centre for Estate Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency, Indonesia. Science Center and Technology Soekarno, Jl. Raya Jakarta-Bogor KM 46, Cibinong, Bogor, Indonesia

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Corresponding Author:
Sesanti Basuki
Research Centre for Estate Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency, Indonesia. Science Center and Technology Soekarno, Jl. Raya Jakarta-Bogor KM 46, Cibinong, Bogor, Indonesia
Email: sesanti.basuki@gmail.com

Abstract: Stevia leaves have the potential to be developed as a natural sweetener, a companion to cane sugar, especially for those who need low-calorie intake. Increasing the productivity of stevia plants is approached by increasing plant biomass accumulation through balanced nitrogen fertilization. This research aims to obtain optimal nitrogen fertilization in the cultivation of stevia plants in which the nitrogen derived from various sources. The experiment was arranged in a factorial Randomized Block Design (RBD) with two factors and three replications. The first factor was organic fertilizer consisting of 4 treatments (C_1: Without *Crotalaria juncea*; C_2: *Crotalaria juncea* 6 ton/ha; C_3: Cow-dung manure 6 tons/ha; C_4: *Crotalaria juncea* L. 3 ton/ha + cow-dung manure 3 tons/ha). The second factor was the dose of N fertilizer which consisted of 4 treatments (D0: 0 kg N ha^{-1} fertilizer; D1: 50 kg N ha^{-1}; D2: 100 kg N ha^{-1}; D3: 200 kg N ha^{-1}). The study result showed that the optimum dose of N fertilization was at a level of N 50 kg ha^{-1}. The leaf weight ratio was higher than the stem weight in all N fertilization treatments. Application of organic material derived from *Crotalaria Juncea* 6 tons' ha^{-1} gave the highest yield of stevia biomass, followed by the application of organic matter with a combination of *crotalaria* 3 tons' ha^{-1} + manure 3 tons' ha^{-1}. The highest leaf ratio was achieved in the treatment of organic matter derived from *Crotalaria juncea*, namely 61% of the total weight of the biomass. The application of organic fertilizer derived from *Crotalaria juncea* would reduce the use of inorganic N fertilizers which leads to reduced stevia farming costs. Moreover, applying a combination of organic and inorganic fertilizers will provide benefits not only for plant growth and yields but for soil health as well.

Keywords: *Crotalaria*, Natural Sweetener, Optimum Fertilization, Productivity, *Stevia*

Introduction

Stevia (*Stevia rebaudiana* bertoni) has the potential to be developed as a natural sweetener by extracting the leaves (Mawarni, 2011), which it can substitute for synthetic sugar. Stevia leaves contain steviol glycosides as the main active ingredient with stevioside as the largest composition (Rafiatul Azkiyah *et al*., 2019). Stevioside is stable at high temperatures (200°C) and does not break down like saccharin or aspartame (Kumar *et al*., 2012). This compound has a sweet taste 300 times stronger than that of sucrose (Yadav *et al*., 2011), but has no calories. Therefore, it does not have a negative influence on obesity and is also safe for diabetics. The compound that provides a sweet taste (stevioside) is synthesized and stored in the leaves, so the need for nutrients that support the growth and development of leaves is very necessary for the cultivation of stevia plants Fig. (1).

Nitrogen is known as a vital compound needed for stimulating vegetative growth in plants including stevia. (Diniz *et al*., 2017) stated that a dose of 5 g urea/plant gave the best results on plant height, leaf area, and dry weight (shoot and root) of stevia plants. Furthermore, (Sawyer, 2004) stated that almost all plants require nitrogen as a regulator of the use of potassium, phosphorus, and other constituent nutrients. Optimum plant growth can only take place if sufficient nitrogen, phosphate, potassium, and other essential elements are available (Ketut *et al*., 2019). Without sufficient nitrogen for growth, stevia plants cannot reach their full growth potential, resulting in lower biomass.

The cultivation technique approach that can be taken to increase biomass yields in stevia plants is to carry out balanced fertilization. Apart from increasing plant biomass accumulation, fertilization can also extend the vegetative phase. By extending this phase, it is expected to increase the dry weight of leaves which will ultimately increase steviol glycosides content. In other words, quality improvement is able to obtained by increasing the partitioning of steviol glycoside compounds that are accumulated in the leaves (Libik-Konieczny *et al*., 2021; Yadav *et al*., 2011).

Rational and balanced fertilization is the application of fertilizers by paying attention to nutrient levels in the soil, type, and quality of fertilizers, as well as considering the nutrients needed by plants for optimum growth and yields (Seufert *et al*., 2012). Providing organic fertilizers is needed to balance the use of inorganic fertilizers so it can maintain or increase soil fertility. In addition, organic matter is one of the ingredients for forming soil aggregates, which has a role as an adhesive between soil particles to unite into soil aggregates, so organic matter is important in the formation of soil structure. Providing a combination of organic and inorganic fertilizers will provide benefits not only for plant growth and yields but for soil health also.

The organic fertilizer could be in the form of green manure derived from plants in which the source of green manure that is often used is *Crotalaria sp*. *Crotalaria* is a genus of flowering plants belonging to the pea family (*Fabaceae*) Fig. (1). It's native to tropical and subtropical regions worldwide. Many species are cultivated as green manure crops, providing nitrogen to the soil, improving soil structure, and suppressing weeds (Baba *et al*., 2019; Assis *et al*., 2024). One of them is *Crotalaria juncea*. Green manure derived from *C. juncea* is decomposed into soil to produce colloids or clay minerals that contain humus and play a role in improving soil properties (Piraneque Gambasica *et al*., 2018). *C. Juncea* has a fairly high nitrogen content of around 3.01%. Apart from the reasons mentioned above, *C. Juncea* plants have root nodules that are able to bind free N from the air up to 60% (Barbosa *et al*., 2020; Hanafi *et al*., 2014). Hence, the incorporation of *Crotalaria* waste into the soil enhances soil structure, water retention, and soil aeration. This leads to healthy status of the soil and improved crop yields.

Fig. 1: Image of *Stevia rebaudiana* and *Crotalaria juncea*
Source: Developed by the authors

This study focuses on obtaining information related to nitrogen fertilization from both organic and inorganic sources not only to increase stevia biomass yields but also to improve soil health and environmental conservation. The information obtained would help the stevia cultivators understand that the use of different types of fertilizer has their benefits and limitations (Fernandus, 2023; Sofyan *et al*., 2019; Sun *et al*., 2020). Furthermore, this finding would be useful for sustainable agriculture practices and may also assist in solving environmental issues associated with excessive use of inorganic fertilizers.

Materials and Methods

Research activities were carried out in January-December 2021 at the Sukapura Experimental Garden, Pasirian-East Java. The area is located at coordinates 7°53'12.4"S 113°02'58.4"E and an altitude of 950 m above sea level. The materials used in this study were *Stevia* stem cuttings and fertilizers (organic and inorganic).

The experiment was arranged in a factorial Randomized Block Design (RBD) with two factors and three replications. The first factor was organic fertilizer consisting of 4 treatments (C_1: Without *Crotalaria juncea*; C_2: *Crotalaria juncea* 6 ton/ha; C_3: Cow-dung manure 6 tons/ha; C_4: *Crotalaria juncea* L. 3 ton/ha + cow-dung manure 3 tons/ha). The second factor was the dose of N fertilizer which consisted of 4 treatments (D0: 0 kg N ha^{-1} fertilizer; D_1: 50 kg N ha^{-1}; D_2: 100 kg N ha^{-1}; D_3: 200 kg N ha^{-1}). Overall, there were 4×4×3 = 48 experimental units. The plot size is 150×300 cm, with the planting distance used being 30×30 cm. P and K fertilizers were given at the same dose for all treatments, namely 100 kg P/ha and 100 kg K/ha.

The treatment of organic fertilizers was mixed evenly on the soil during the last tillage/bed formation. TSP and KCl fertilizers were given as basic fertilizer at the time of planting, while urea fertilizer was applied at the age of 30 Days After Planting (DAP) half dose, and the rest was given at the age of 60 DAP. Plant maintenance consists of irrigation, pest and disease control, and weed control adjusted to conditions in the field and refers to stevia cultivation standards. Parameters observed included growth parameters (plant height, number of branches, canopy width, flowering age, fresh weight of herbs). Propagation of plant material was carried out at Karangploso Experimental Garden, Malang-East Java. Planting of *stevia* seedlings was carried out in May 2021, starting with planting *Crotalaria* first. Planting *Crotalaria* as a source of organic material was carried out at the same time as soil tillage by planting them along the embankments. *Crotalaria* was harvested just before the flowering phase Figs. (2-4).

Temperature Stress Treatments

Pollen Germination

Fig. 2: Preparation of stevia plant material at karangploso experimental garden and land preparation for the optimum fertilization of stevia research activity at sukapura experimental garden

Fig. 3: Application of *Crotalaria* and planting in the optimum fertilization activities for stevia at the sukapura experimental garden

Fig. 4: Maintenance of optimum fertilization activities for stevia at sukapura experimental garden

Results and Discussion

The observation of stevia vegetative phase at 8 Weeks After Planting (WAP) showed that the highest stevia growth performance (plant height, canopy width, number of branches, longest branch) was found at 50 kg N ha-1 inorganic fertilizer level. Increasing the fertilizer dose from 50-100 kg N ha-1 and up to 200 kg N ha-1 reduces plant growth performance (Fig. 5). The research result indicates that the optimal growth performance of stevia plants observed at 8 WAP was provided by plants fertilized with an inorganic fertilizer level of 50 kg N ha-1. Increasing the dose of N fertilizer from this level not only caused a decrease in growth performance but triggered the flowering of stevia plants also Fig. (5). The results of this study are in line with the general principle of plant physiology that balanced nutrient application is crucial for maintaining optimal plant growth and development. It can be stated that increasing N fertilization will generally enhance plant growth, however, excessive use of N results in reducing plant growth and promoting the flowering process. As the plants will shift from the vegetative phase to the generative phase (Junaidath *et al*., 2022).

Further observations using organic material treatment on plant growth performance when the plants were 8 WAP shows that there was no difference between organic matter source treatments on stevia plants' growth performance (plant height, canopy width, number of branches, longest branch) (Fig. 6). This is thought to be related to environmental conditions when the research was carried out. The activities are carried out during the dry season, from planting to harvesting, in which the intensity of sunlight and temperature is quite high. Actually, the nature of the organic material tends to increase the soil's water-holding capacity which allows the soil to retain more water. However, apart from having the ability to store water, under certain circumstances, organic material can compete with plant roots to absorb water in the soil. High organic matter content in the soil can bind water tightly, thereby reducing water available to plant roots (Carter, 2002; Youssef *et al*., 2021). In line with (Junaidath *et al*., 2022) study which stated that the addition of organic matter into the soil can compete with plant roots for water absorption. Moreover, (Cates, 2020; Luke Gatiboni, 2022) stated that the addition of organic matter that has the property of binding water in the soil will affect the availability of water for plants which will ultimately inhibit plant growth. In such conditions, it is often followed by accelerating flowering (Fig. 6).

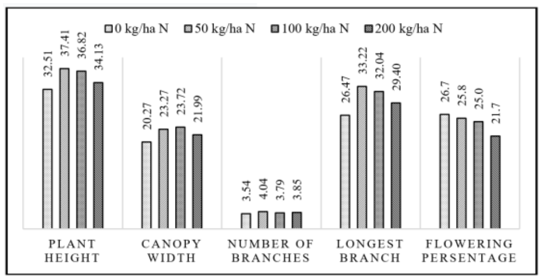

Fig. 5: Plant height, canopy width, number of branches, longest branch, and flowering time at various levels of nitrogen fertilizer

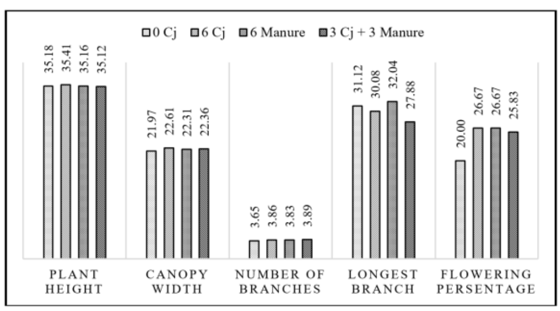

Fig. 6: Plant height, canopy width, number of branches, longest branch, and flowering time on various sources of organic matter

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Environmental conditions, such as water availability and nutrients, are one of the factors that influence plant growth and yields besides genetic factors (Jarma-Orozco *et al*., 2020; Pal *et al*., 2015) stated that dry leaf yield and accumulation of stevioside compound in stevia plants are controlled by environmental factors and agronomic management. Thus, the leaf yield and secondary metabolite profile of stevia are able to be improved through the proper selection of growing sites and proper nutritional management. Our study result shows that the addition of N fertilizer from 0 kg N ha^{-1} (without N fertilizer) to 50 kg N ha^{-1} increased the stevia plant yields in the form of plant biomass. If the dose of N fertilizer was increased from 50 kg N ha^{-1} to 100 kg N ha^{-1} and up to 200 kg N ha^{-1}, the stevia plant yields declined (Fig. 7). The study result indicates that the optimum dose of N fertilization for stevia plants in the Sukapura Experimental Garden was at the N level of 50 kg ha^{-1}. Although plants require a balanced diet of nutrients, excessive nitrogen can disrupt this balance, leading to deficiencies in other essential elements such as potassium, phosphorus, and magnesium (Xu *et al*., 2020; Yousaf *et al*., 2021). These elements are crucial for various plant processes, including photosynthesis, energy production, and cell wall formation. In other words, an imbalance the nutrients can hinder overall plant growth and development. In addition, according to (Rivera-Avilez *et al*., 2021) the excessive N application will shift the plant growth phase to the flowering phase where the plant's energy is diverted towards the generative phase rather than vegetative development. Due to stevia is cultivated primarily for its sweet-tasting compounds (steviosides) which are concentrated in the leaves, hence, the excessive nitrogen that would promote flowering will ultimately reduce stevioside yields.

The nutritional dose for Stevia can vary based on the environment and soil type. This flexibility allows Stevia to thrive in different conditions. Under average climatic conditions and soil type, a recommended nutrient application is approximately 70 kg of Nitrogen (N), 35 kg of Phosphorus (P), and 45 kg of Potassium (K) per hectare (Chughtai *et al*., 2019). The growth parameters such as plant height and stem diameter were significantly higher with a treatment of 200 N, 150 P, and 80 K. Conversely, the stevioside and total steviol glycoside contents were higher in a treatment of 100 N, 100 P, and 240 K (Benhmimou *et al*., 2018).

The purpose of fertilization is to add deficient plant nutrients to the soil because these nutrients are necessary for plant growth. The observation result shows that the stevia leaf weight ratio was higher than the stem weight ratio in all N fertilization treatments Fig. (8).

This indicates that the nutrient content of the stevia leaves and/or metabolic activity in the leaves is higher than that of the stems. The result is thought to be related to stevia as a plant that produces stevioside compounds which are processed in its leaves, so the distribution of nutrient requirements to leaves tissue to support the leaf's growth and development is prioritized by the plant.

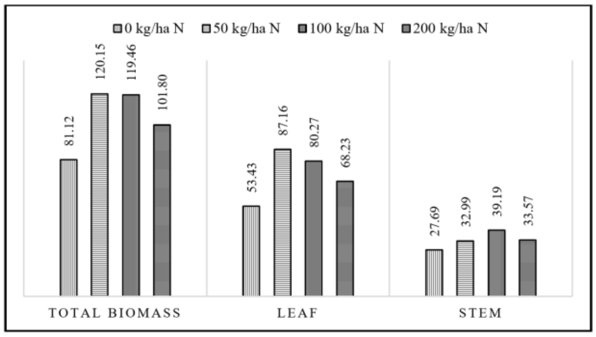

Fig. 7: Total weight of biomass, weight of leaves, and weight of stems at various levels of nitrogen fertilizer

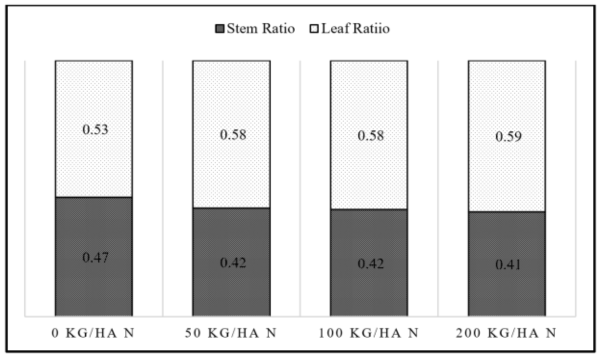

Fig. 8: Ratio of leaf weight and stem weight at various levels of nitrogen fertilizer

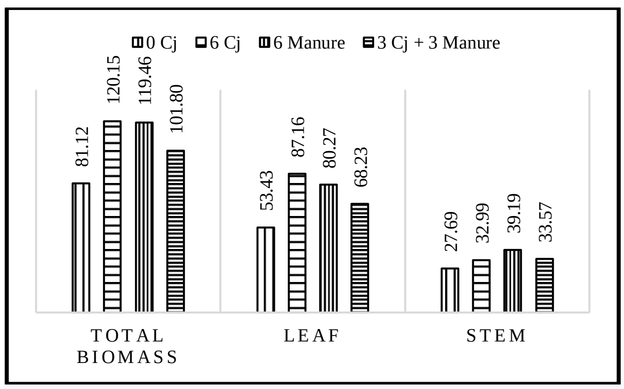

Fig. 9: Weight of biomass, weight of leaves, and weight of stems on various sources of organic matter

In order to achieve high biomass yields of stevia plants, optimal fertilization support is needed, not only in terms of the amount of fertilizer applied, but the source of fertilizer also needs to be considered. The data obtained from the study shows that the application of organic materials derived from *Crotalaria* and in the form of manure significantly impacts stevia biomass yields and leaf-to-stem weight ratios Fig. (9). Organic fertilizer derived from *Crotalaria* 6 tons ha^{-1} provided the highest stevia biomass yields, followed by the treatment of giving manure 6 tons ha^{-1}, then by giving a combination of both fertilizers. Organic fertilizer derived from *Crotalaria* 6 tons ha^{-1} provided the highest stevia biomass yields, followed by the treatment of giving manure 6 tons ha^{-1}, then by giving a combination fertilizer of 3 tons ha^{-1} manure +3 tons ha^{-1} *crotalaria*, while, the lowest stevia biomass yields were in the treatment without the addition of any organic matter (Fig. 9). Providing a combination fertilizer of 3 tons ha^{-1} manure +3 tons ha^{-1} *Crotalaria* also shows a notable increase in stevia biomass yields. The study result indicates that the combination of organic materials from different sources provides a synergistic effect that has the potential to improve soil fertility by enhancing nutrient availability which will promote better plant growth and ultimately lead to increased biomass yields. These findings underscore the importance of adopting sustainable agricultural practices that prioritize soil health for long-term crop productivity (Baba *et al*., 2019; Calapardo and Manigo, 2024; Enchev *et al*., 2018).

In general, the ratio of leaf weight was higher than stem weight in all treatments with organic matter. The highest leaf ratio was observed when the organic matter derived from *Crotalaria juncea* at 61% of the total biomass weight (Fig. 10). This indicates that the organic matter not only increases overall biomass yield but also promotes leaf growth more significantly than stem growth. According to (Baba *et al*., 2019), the application of organic fertilizers is generally used to balance the use of inorganic fertilizers in order to improve soil fertility. This statement agrees with the research results of (De Assis *et al*., 2024; Fernandus, 2023) which stated that the addition of green manure derived from C. juncea 25 tons ha-1 has reduced the use of inorganic fertilizers by 50%, while, the addition of green manure derived from C. mucronata 25 tons ha-1 has reduced the use of inorganic fertilizers by 25%. Compared to organic fertilizer derived from livestock manure, green manure derived from plants, such as crotalaria, has several advantages, including the composition of N content and organic material produced at several stages of its growth. The maximum nitrogen content in *C. juncea* plants occurred before the beginning of the flowering period. C*rotalaria* plant contained 5.25% N and 69.55% organic matter at the age of 14 days after planting (dap), furthermore, it contained 4.29% N and 66.85% organic matter at the age of 30 dap. While, at the age of 42 days after planting it contained 2.49% N and 66.78% organic matter (Ali *et al*., 2016). As plants mature phase, they prioritize nutrient allocation towards reproductive organs like flowers and seeds In addition, crotalaria is easy to grow in various soil conditions, fast growth rate, large biomass production, and a fast decomposition process (Baba *et al*., 2019). Organic materials that have been decomposing well, not only enrich nutrition for plants but also play a major role in improving soil properties. Applying green fertilizer to the soil is one way to maintain soil fertility (Curto *et al*., 2015).

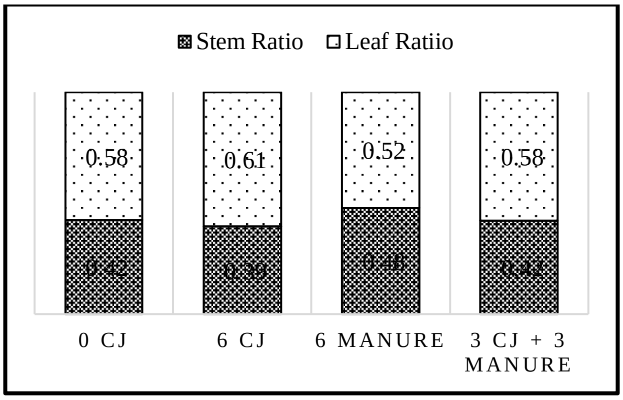

Fig. 10: Ratio of leaf weight and stem weight at various sources of organic matter

Our study result reveals the crucial role of nitrogen in enhancing the growth and productivity of stevia plants. The absence of nitrogen fertilizers resulted in poorer growth parameters, as well as the lower total biomass yielded. In addition, the results discussed above show that the application of organic materials as a source of N such as *crotalaria* and manure resulted in a higher leaf-to-stem weight ratio and significantly increased stevia biomass yielded. According to (Youssef *et al*., 2021), organic nitrogen improves soil properties such as Soil Organic Carbon (SOC), soil structure, and available nutrients in which these improvements contribute to better nutrient uptake by the plant over a longer period, promoting healthier plant growth (Tripatmasari *et al*., 2021). Finally, our findings support the use of sustainable agricultural practices that prioritize soil health and organic amendments for optimal plant growth and productivity.

Conclusion

Nitrogen plays a crucial role in enhancing the growth and productivity of stevia plants. The optimum dose of N fertilization for stevia at Sukapura Experimental Garden was at a level of N 50 kg ha^{-1}. In general, the leaf weight ratio was higher than the stem weight in all N fertilization treatments. The highest leaf ratio was achieved in the treatment of organic matter derived from *Crotalaria juncea* which was 61% of the total weight of biomass. Application of organic matter derived from *Crotalaria* 6 tons ha^{-1} gave the highest production of stevia biomass, followed by the application of organic matter treatment with a combination of *Crotalaria* 3 tons ha^{-1} + manure 3 tons ha^{-1}.

For future studies, we recommend implementing the combination of manure and *Crotalaria* as a standard practice for optimizing stevia leaf production. This method not only increases biomass yield but also enhances the leaf weight ratio, which is essential for high-quality sweetener production. Furthermore, farmers should start fertilizing using organic materials to minimize the use of inorganic fertilizers such as urea. Through this method, farmers can save costs on stevia farming and take an active role in sustainable agriculture, as well as increase the yield and quality of stevia in Indonesia so that stevia is able to compete in the global market.

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Author's Contributions

Mohammad Cholid: Responsible for conducting research, collecting data, and preparing journal manuscripts.

Sesanti Basuki: Supervise research implementation, collecting data, revising and perfect journal manuscripts.

Budi Hariyono, Fitriningdyah Tri Kadarwati and Prima Diarini Riajaya: Supervise research implementation, revise and perfect journal manuscripts.

Ethics

This article contains research results that have not yet been published. The corresponding author declares that there are no ethical issues and that the other authors have read and agreed on the manuscript for publication.

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