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Enhancing Herbage Growth, Yield and Quality of Stevia (*Stevia rebaudiana* Bertoni) using Bio-Organic Nutrients in Varied Soil Media

(Meningkatkan Pertumbuhan Herba, Hasil dan Kualiti Stevia (*Stevia rebaudiana* Bertoni) menggunakan Nutrien Bio-Organik dalam Pelbagai Media Tanah)

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ABSTRACT

The present method of stevia propagation is still insufficient to meet its increasing demand. Moreover, the use of organic inputs as substitutes for inorganic fertilizers to improve soil quality and productivity is also desired. In this study, the effects of bio-organic nutrients and different growing media compositions on Stevia's herbage growth, yield and quality were determined. A completely randomized design was used with 20 rooted stem cuttings per replication at one cutting per potting bag, laid in a 4 × 4 factorial arrangement. Growing Media (garden soil alone [GS]; GS + rice hull [RH]; GS + vermicompost [VC]; and GS+RH+VC) served as factor A while bio-organic nutrients (fermented fruit juice [FFJ]; vermi tea [VT]; seaweed tea [ST] and control) as factor B. There were 16 treatment combinations replicated four times. Results showed that GS+RH+VC consistently improved the survival rate, plant height, leaf and shoot count per plant, weight (fresh and dry), and herbage yield of Stevia. Furthermore, VT boosted the herbage yield and visual quality. In terms of interaction effects, plants grown in garden soil mixed with vermicompost (GS+VC) with VT application developed more leaves than other treatment combinations. Hence, these bio-nutrients and growing media composition significantly enhanced the production of Stevia.

Keywords: Herbage yield; organic agriculture; Stevia propagation; Vermi tea

ABSTRAK

Kaedah pembiakan stevia pada masa ini masih tidak mencukupi untuk memenuhi permintaannya yang semakin meningkat. Selain itu, penggunaan input organik sebagai pengganti baja bukan organik untuk meningkatkan kualiti dan produktiviti tanah juga diingini. Dalam kajian ini, kesan nutrien bio-organik dan komposisi pertumbuhan media yang berbeza terhadap pertumbuhan herba, hasil dan kualiti Stevia telah ditentukan. Reka bentuk rawak sepenuhnya telah digunakan dengan 20 keratan batang berakar setiap replikasi pada satu keratan setiap beg pasu, diletakkan dalam susunan faktorial 4 × 4. Media penanaman (tanah taman sahaja [GS]; GS + sekam padi [RH]; GS + vermikompos [VC]; dan GS+RH+VC) berfungsi sebagai faktor A manakala nutrien bio-organik (jus buah yang ditapai [FFJ]; teh vermi [VT], teh rumpai laut [ST] dan kawalan) sebagai faktor B. Terdapat 16 gabungan rawatan diulang empat kali. Keputusan menunjukkan bahawa GS+RH+VC secara tekal meningkatkan kadar kemandirian, ketinggian tumbuhan, bilangan daun dan pucuk setiap tumbuhan, berat (segar dan kering) dan hasil herba Stevia. Tambahan pula, VT meningkatkan hasil herba dan kualiti visual. Dari segi kesan interaksi, tumbuhan yang ditanam dalam tanah taman bercampur dengan vermikompos (GS+VC) dengan penggunaan VT menghasilkan lebih banyak daun berbanding gabungan rawatan lain. Oleh itu, bio-nutrien dan komposisi penanaman media meningkatkan pengeluaran Stevia dengan ketara.

Kata kunci: Hasil herba; pembiakan Stevia; pertanian organik; teh Vermi

INTRODUCTION

The contemporary surge in the consumption of food products containing high levels of sugars and artificial sweeteners nowadays has led to the proliferation of chronic ailments among people. Notably, diabetes and obesity have emerged as the two primary disorders associated with the excessive intake of high-calorie sugars (Singh, Mehrotra & Tiwari 2014). In the Philippines, reports showed that 6 million Filipinos have been diagnosed with diabetes (Philippine Daily Inquirer 2016) and this contributes to a national prevalence rate of 4.1%, which is higher in urban areas compared to rural ones (Jimeno et al. 2015). This prevalence has witnessed an alarming increase from 7.2% to 10.2%, with a higher incidence among women, reaching 5.8% across all age groups. Additionally, there is a documented mortality rate of 0.02% in the age group of 30-69 and 0.01% in individuals aged 70 and above (American Diabetes Association 2009). The detrimental impact on human health has propelled the global stevia market, fueled by the escalating demand for natural sweeteners in the food and beverage industry and the growing consumer preference for low-calorie alternatives (Fior Markets 2020).

In an effort to mitigate the rising incidence of diabetes, one approach involves the adoption of natural plant alternatives such as stevia. The herb stevia is a non-caloric natural sweetener that is sweeter than sugar about 300-350 times, stable at high temperatures, stain-free, and suitable for human ingestion. Steviol glycosides from stevia offer a solution for the prevention of complex diabetic problems and obesity (Singh, Mehrotra & Tiwari 2014). Consistent use of these non-caloric and non-carcinogenic sweeteners extracted from *Stevia rebaudiana* leaves has been associated with potential benefits. These include the potential to lower blood cholesterol levels, radionuclide levels, and sugar levels, leading to improvements in blood coagulation and cell regeneration. Furthermore, there is evidence suggesting that stevia may contribute to inhibiting tumor growth and promoting the firming of blood arteries (Barriocanal et al. 2008).

Furthermore, stevia is a potential alternative or substitute for cane sugar. Its leaves not only offer sweetness but also contain carbohydrates, proteins, crude fiber and other nutrients, making it a good substitute for sugar and ingredients in processed foods and beverages (Gasmalla, Yang & Hua 2014). Unlike some artificial sweeteners, stevia has no adverse effects on blood sugar levels, kidneys, or the nervous system, contributing to its reputation as a safe option. Moreover, stevia possesses anti-fungal and anti-bacterial qualities, making it safe to use in toothpaste, mouthwash, and tonics for diabetes patients. Additionally, the leaves of stevia can be brewed as a tea and has proven to be excellent in soothing an agitated stomach (Goyal, Samsher & Goyal 2010).

The potential of stevia caught the attention of herbal plant propagators and researchers worldwide. However, the current method of stevia propagation is still insufficient to meet the current rapid increase of demand for its planting materials. Stevia plants are primarily propagated through vegetative stem cuttings, yet this method yields low outputs with limited success rates. The most popular way to provide plants the nutrients they need is to apply conventional soil fertilizer. However, absorption of micronutrients is more efficient when applied by foliar sprays at an appropriate concentration. Foliar fertilizer have proven effective in addressing nutritional disorder in plants, offering ecological safety and economic affordability (Fageria et al. 2009).

Substituting inorganic fertilizers with bio-fertilizers enriched bio-organic nutrients, offers a promising approach to enhance soil quality. The application of organic fertilizers not only fosters the production of high-yielding planting stock material but also reduces input costs, particularly in developing countries. Through organic processes, bio-fertilizers enhance the quantity and accessibility of both macronutrients and micronutrients, thereby promoting plant development (Vessey 2003).

According to Das et al. (2007), the application of bio-fertilizer on stevia has been found to enhance both the growth and herbage yield of the plant. This improvement is attributed to the bio-fertilizers' capability to fix atmospheric nitrogen both symbiotically and non-symbiotically, along with their ability to convert essential nutrients in the soil (such as P, Zn, Cu, Fe, and S) from fixed or non-usable forms to usable forms. Additionally, bio-fertilizers contribute to the decomposition of organic wastes through biological processes. Consequently, the stevia plant experiences increased biomass production, facilitated by the delivery of nutrients in forms that are easily absorbable by the plant.

There is a myriad sources of organic nutrients that can be utilized in organic farming. Fertilizers made from natural organic materials and fruit waste have several advantages for both soil and plants. Due to the high nutrient content and microbial diversity in fermented fruit waste which feeds nutrients to crops, it is considered valuable in organic farming (Alkorta et al. 1998). A synthetic honey called fermented fruit juice (FFJ) is derived from sweet ripe fruits, vegetables, and root crops. These ingredients are thoroughly mixed with molasses before being fermented. FFJ can be readily absorbed by plants due to its natural enzymes (Badar & Qureshi 2012). It works as a floral inducer, fruit setter, and accelerator of soil microbial activity. Farmers have observed its effectiveness when applied to leaves at a rate of 2 to 4 tablespoons per gallon of water, starting from a crop's flowering phase through the fruit set. FFJ derived from ripe fruits contain essential elements such as phosphorus and potassium, crucial for flowering and fruit (Agricultural Training Institute 2011).

Vermicomposting is another promising technology that can benefit farmers by improving their productivity. Various vermi technologies, including vermicompost and vermi tea, have been studied extensively by many researchers and scientists, demonstrating varying degrees of success in vegetable production. Vermi tea, a liquid extract derived from vermicast, is meticulously brewed to cultivate beneficial microorganisms. This tea is then applied to both the soil and leaves of plants to foster growth and vitality. The extracts contain essential nutrients, including nitrate or ammonium in nitrogen form, phosphorus, potassium, and a diverse array of microorganisms. These components prove beneficial in aiding plants to recover from the impact of pesticides and other environmental stresses. According to Badar and Qureshi (2012), appropriately processed organic wastes, such as compost, can produce a sufficient source of food and energy for local microorganisms, particularly those that are rhizosphere-competent. Based on laboratory analysis, a vermicompost tea prepared at a 1:10 ratio contains an average nitrate (NO_3) level of 77 ppm, ammonium (NH_4) levels of 3.7 ppm, P levels of 18 ppm, and K levels of 186 ppm (Balfanz 2011).

On the other hand, the utilization of seaweeds and its derivatives which offers a variety of substances that promote plant growth, are also employed as additions to crop production systems. As natural and sustainable resource, liquid seaweed fertilizer stands out for providing a wide range of nutrients that can benefit all types of plant growth. It contains important hormones like auxins, cytokinins, betaines, and gibberellins essential to plant health (McHugh 2003). This liquid seaweed fertilizer is a fermented extract from seaweeds which contains higher concentration of chlorophyll. It is abundant in traces of nitrogen, magnesium, potassium, zinc, iron, and other elements (Yao et al. 2020). This natural growth enhancer is used for soil and crop treatments (Khan et al. 2009) because it is rich in micro and macronutrients vital for plant growth and development, as well as helps in improving soil fertility. Studies on seaweed extracts showed that they possess an ability to enhance and improve growth and yield by increasing crop mineral absorption (Khan et al. 2009). It has also been proven that seaweeds contain a wide variety of organic compounds, including antioxidants, hormones for plant growth and mineral nutrients (Pacholczak, Nowakowska & Pietkiewicz 2016).

An ideal growing media is defined by several key attributes. It should effectively support the plant structure, ensuring it is held securely in space. Additionally, the medium must supply adequate moisture, aeration and essential nutrients for optimal plant growth. An essential consideration is the absence of weed seeds and harmful microorganisms, while actively fostering a beneficial microbial population that protects the plants against nutrient pests and diseases. Moreover, the medium should be sterilizable without compromising nutrient integrity. A high-quality growing medium should embody a well-balanced combination of porosity, exemplified by components like rice hulls and sand, and organic matter such as vermicast. This blend not only ensures proper air and water circulation but also maintains the friability required for the successful germination and rooting of stem cuttings. Nevertheless, considerations regarding the availability, cost, and ease of procurement of these components are paramount (Haefele et al. 2011).

To achieve higher yield and improved quality in stevia production, various factors play a crucial role. The application of diverse bio-organic nutrients and the utilization of different growing media have been hypothesized as potential strategies to enhance the herbage growth, yield, and quality of stevia. Additionally, addressing the surging demand for stevia presents an opportunity to meet public expectations for organic inputs in plant production for human consumption. The overarching goal of this experiment is to investigate the impact of applying bio-organic nutrients on the herbage growth, yield, and quality of stevia grown in various growing media compositions. The primary focus is to assess the individual effects of bio-organic nutrients and growing media, as well as to explore the potential interaction effects between these two crucial factors. By doing so, the study aims to contribute valuable insights into optimizing stevia cultivation practices for enhanced productivity and quality, aligning with the growing interest in organic approaches in plant production.

MATERIALS AND METHODS

LOCATION AND MATERIALS USED

The experimental setup was established at the Davao Oriental State College of Science and Technology- Cateel Campus, Mahan-ob, Mainit, Cateel, Davao Oriental from August 2019 to December 2019. Rooted cuttings of stevia were used as experimental materials obtained from Umanica Farm, Malaybalay City, Bukidnon, Philippines. The growing media such as vermicast and composted rice hull were purchased from Taytayan Irrigation Association (TIA), Barangay Taytayan, Cateel, Davao Oriental, Philippines. Fish nets, polyethylene bags (PEB), hose, meter stick, blanch polyrope, digital weighing scale and other materials were purchased from the local agricultural outlets.

EXPERIMENTAL DESIGN AND TREATMENTS

Rooted stem cuttings of stevia were planted in PEB and grown under a screened nursery. The experimentation was laid in 4 × 4 factorial arrangements in completely randomized design (CRD) with 20 rooted stem cuttings per replication at one cutting per PEB. Growing media served as factor A with four variables such as control or garden soil alone (GS); garden soil (GS) + rice hull (RH); garden soil (GS) + vermicompost (VC); and GS+RH+VC while the factor B was the bio-organic nutrients consists of control or no application; fermented fruit juice (FFJ); vermi tea (VT); and seaweed tea (ST). There were 16 treatment combinations which were replicated four times.

The stevia-planted bags were arranged on elevated plots with waterproof vinyl tarp lining to separate the different treatments. The nursery was also covered with fish nets to avoid entry of stray animals. The total experimental area of 14 m × 14 m (196 m^2) was divided into 64 experimental plots of equal sizes with an alleyway of 0.50 m between plots. However, periphery of the nursery was also planted with stevia as border plants.

PREPARATION OF GROWING MEDIA

Garden soil, vermicast, and rice hulls were used as growing media. Media mixtures of garden soil and either vermicast and/or rice hulls were mixed proportionally based on parts (1:1). Polyethylene bags (PEB of 14 × 16” × 0.0002) with a capacity of 5 kilograms growing media were used. Each PEB was filled with up to the brim uniformly. Garden soil was solar sterilized prior to mixing while no sterilization was imposed on other growing media.

PREPARATION OF BIO-ORGANIC NUTRIENTS

Bio-organic nutrient sources used in this study were FFJ, VT and ST. Fermented extracts were stored for three days before use. For FFJ, the ingredients used were ripe fruits of banana (*Musa acuminata* [AA Group] ‘Lakatan’), papaya (*Carica papaya*), mango (*Mangifera indica*), squash (*Cucurbita maxima*), and tubers of sweet potato (*Ipomoea batatas*). These were chopped into small pieces, with each component totalling one kilogram, and were added with molasses at 1:1 ratio, and thoroughly mixed. Fermentation process took seven (7) days and extracts were collected immediately, placed in clean bottles and stored at room temperature.

On the other hand, VT was prepared by taking one kilogram of worm castings, enclosed in a sackcloth bag, which was then tied at its neck. The bag was submerged in an aquarium containing 2 L of water for 24 h, assisted by an aerator. The resulting extract was added with 200 mL of molasses as vermi stock. The vermi stock was further diluted at 1:2 ratio, with one part being water, to constitute the vermi tea was then sprayed onto the stevia plant.

For ST, the process involved fermenting one kilogram of fresh seaweeds in one liter of water (1:1 ratio) for seven days. Stirring of the mixture was done at two-days interval. The resulting extracts were considered as stock solution. One liter of water was used to dilute each 20 mL of stock solution before being applied to stevia plants.

SOIL AND BIO-ORGANIC NUTRIENT ANALYSIS

Composite soils samples (garden soil, composted rice hulls, and vermicast) and bio-organic nutrient samples were submitted to the Soil and Plant Analyses Laboratory (SPAL) of Central Mindanao University (CMU), Musuan, Bukidnon for chemical analysis before and after the conduct of the experiment. Soil pH was determined via the potentiometric method in a supernatant suspension with a 1:2.5 soil-to-water ratio, following the protocol outlined by Peech (1965) and utilizing a digital pH meter. Soil organic matter content was assessed through the Walkley-Black method, which involves wet oxidation with dichromate in concentrated H_2SO_4, with 1M $K_2Cr_2O_7$ serving as the primary heat source (Nelson & Sommers 1982). Available phosphorus (P) levels were determined using the P-Bray II extraction method (Bray & Kurtz 1945), while exchangeable bases like potassium (K) were analyzed via the 1N NH_4OAC at pH 7 method (Metson 1956), employing microwave plasma-atomic emission spectrometry (MP-AES) 4200.

On the other hand, bio-organic nutrient analysis involved the Kjeldahl Method for nitrogen percentage determination, entailing digestion, distillation, and titration (Bremner 1960). Phosphorus (%P) content was quantified through Colorimetric Methods using a Colorimeter/Spectrophotometer and measured spectrophotometrically. Potassium (K), calcium (Ca), magnesium (Mg), and copper (Cu) percentages were assessed using Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma (ICP), AA-7000 method, with the organic matrix digested with acids prior to analysis.

TRANSPLANTING OF STEVIA

The planting materials were standardized before transplanting ensuring that only those with uniform size, height, and the same number of leaves (4-5 true leaves) per cutting were used. To achieve uniformity, some cuttings were defoliated, resulting in a consistent initial number of leaves. A total of 640 rooted cuttings were transplanted individually in PEB per plot. Each polybag was watered using a sprinkler before planting to avoid washing-out of rooted cuttings. Samples were labelled according to their respective treatments.

APPLICATION OF BIO-ORGANIC NUTRIENTS

Spray solutions containing bio-organic nutrients (FFJ, VT and ST stock) were prepared by mixing 20 mL of each nutrient treatment in one liter of water. To maintain consistency, each plant received precisely five (5) mL of bio-fertilizers per application. A total of 250 mL of spray solution was applied to each plant using a hand sprayer with a three-liter capacity. The sprayer was modified with calibration marks to ensure accurate 250 mL sprayed to each plant. Additionally, a waterproof vinyl tarp was used to cover neighbouring plant samples and control drifts of bio-organic nutrients during the application process. Application started from 15 DAT at seven days interval up to 75 DAT. Treatments were applied early in the morning since stomates are still open and facilitate nutrient absorption.

CULTURAL MANAGEMENT AND PRACTICES

To avoid water stress and the degeneration of the plants, watering was done every day or as needed. Uniform quantity of water per plant was used during watering. A calibrated can was used starting from transplanting with 50 mL per plant and was increased as the plants grew up to 500 mL per grown plant at 60 and 75 DAT. Weeding and sanitation were done regularly as needed. Hand pulling of weeds was done for all treatments. The clean culture was observed by clearing the entire periphery of the nursery aside from those within the experimental area. Pest monitoring was done regularly. It was observed that only few symptoms of insect pest and diseases occurred, except for leaf curling. No application of pesticide was employed during the study. In terms of herbage harvesting, pinching of flowers was done starting at 30 DAT for stevia to have more bushy leaves. Harvesting was done prior to flowering and started at two months (60 DAT) with one-month interval in two harvesting cycles. This was done by cutting the stems leaving at least four to five inches above the soil surface using a pruning shear. The herbage yield taken from ten samples per treatment per replication was weighed using a digital weighing scale. This was done immediately every after harvest.

DATA COLLECTED

Expressed in percentage (%), the survival rate was measured by recording the number of plants that survived at 15 days after transplanting (DAT). The data were obtained from 20 plant samples per replication per treatment by dividing the number of plants survived over the total plants transplanted multiplied with 100. The plant height on the other hand was gathered by measuring the height of the sample plants per replication per treatment from the base up to the end part of plants using a meter stick. Leaf and shoot count of stevia plant per replication per treatment were determined manually. Incidence of pests and diseases on stevia plants were also monitored daily. For fresh weight, fresh leaves taken from the sample plants per replication per treatment were separated manually from the stalks/stems and were weighed using digital weighing scale and expressed in grams. For dry weight, harvested fresh leaves from each treatment were air dried. Dried leaves taken per replication per treatment were weighed using digital weighing scale and expressed in grams. For herbage yield, harvested herbage (with leaves attached on stems) taken from 10 sample plants per replication per treatment were weighed and expressed in grams. Harvested yield during the first and second harvest were combined and reflected as total yield. During harvesting, the stalks/stems were cut leaving about five inches above the soil level. The fresh herbage yield was assessed using below formula:

$$\text{Fresh herbage yield (g)} = \frac{\text{Total herbage weight (g)}}{\text{Plot area (sq m)}} \text{ x } 100$$

For the visual quality rating (VQR) of leaves, fresh leaves of stevia were rated based on their visual appearance. Visual quality rating of stevia leaves was measured per replication per treatment after harvest using a modified hedonal rating scale of 1-5 with corresponding quality rating and description based on per color description of leaves. Five (5) for excellent - leaves are dark green with a long stalks and fully expanded leaves; four (3) for good - leaves are green with a medium length of stalks with full expanded leaves; and one (1) for poor - leaves are light green with short stalks and partially expanded leaves.

STATISTICAL ANALYSIS

Utilizing the Statistical Tool for Agricultural Research (STAR), the data gathered in this experiment were evaluated through the Analysis of Variance (ANOVA), and the mean differences between treatments were examined using the Tukey's Honest Significant Difference (HSD) test at 5% ($p \leq 0.05$) level of significance (Gulles et al. 2014).

RESULTS AND DISCUSSION

NUTRIENT ANALYSIS OF GROWING MEDIA AND BIO-ORGANIC NUTRIENTS

Presented on Table 1 is the nutrient analysis of growing media before and after the experiment. The pH of the garden soil was measured at 5.70. However, it was noted that when the garden soil was mixed with rice hulls, and vermicasts, the pH increased to 5.80 and 6.14, respectively. Likewise, % OM was least in garden soil but increased specially when added with RH and VC (9.20%). Garden soil has the least extractable Phosphorus (P) and exchangeable Potassium (K). Vermicast has shown to be extremely high in exchangeable K. After the experiment, extractable P and K increased while % OM and pH decreased.

The drop in the percentage of OM may be caused by crop fertilizer loss and organic matter decomposition. This is because as organic acids are formed soil pH decrease especially if the organic material is low in base forming cations. The increase of P and K may be attributed to the organic compounds from organic materials released gradually. Moreover, there are still organic materials in the growing media, hence, a symbiotic relationship between microorganisms and colonies produced by plants on the outside part of the root system may have increased the water and nutrient uptake. This action might take place as a result of the humic acid's functional groups' capacity to interact and react negatively with positively charged ions (Mulyani et al. 2017). Organic materials enhance physico-chemical and biological processes and provide carbon (C) as source of energy for microbial activity, which in turn increases plant growth, development, and output, according to Umesha et al. (2011).

Bio-organic fertilizers are viewed as a potent substitute for reducing the usage of various synthetic fertilizers, which are not only expensive but also have detrimental impacts on the health of the soil (Kumar & Upadhyaya 2012). Table 2 presents the nutrient analysis of different bio-organic nutrients as treatment.

Results showed that FFJ contained relatively high % P. However, the Seaweed tea had relatively higher % K, % Ca and % Mg while Vermi tea showed the least nutrient contents among bio-organic treatments. According to a study by Kumar and Upadhyaya (2012) seaweed bio-fertilizers hold macronutrients (such as Calcium, Magnesium, Phosphorus, and Potassium), micronutrients (such as Iron, Copper, Zinc, Boron, Manganese, Cobalt, and Molybdenum), as well as a variety of plant growth controllers that either enhance crop production or have unswerving influence on plant growth and development.

SURVIVAL RATE (%)

The survival of stevia stem cuttings was highly affected by the growing media and bio-organic nutrients as illustrated in Figure 1. Likewise, significant interactions ($p \leq 0.05$) between these two factors were found at 15 DAT (Table 3). Results of the statistical analysis ($p \leq 0.05$) showed that stevia grown in garden soil alone (control) had the least survival rate of 95.63% which differed significantly from the rest of the plants grown in different media which exhibited survival rates ranging from 99.38 to 100%. On the other hand, the application of bio-organic nutrients on stevia significantly increased the survivability of plants ($p \leq 0.05$) from 99.38% to 100% compared to the control plants which had only 95.63%.

TABLE 1. Nutrient analysis of growing media before and after the experiment (Source: Soil Analysis Result, SPAL, Central Mindanao University, Musuan, Bukidnon, Philippines)

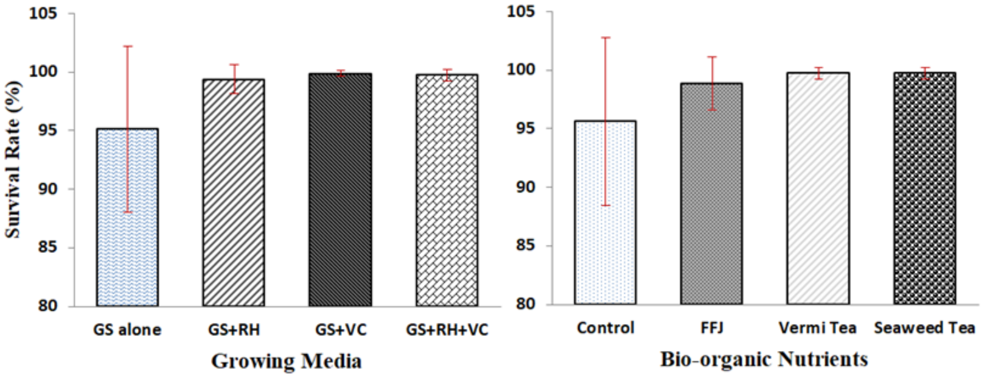
Growing media	Nutrient contents			
	pH	% OM	Exch. P (ppm)	Exch. K (ppm)
Before				
Garden Soil (GS)	5.70	1.29	19.647	108
GS + Rice Hull (RH)	5.80	7.82	42.372	117
GS + Vermicompost (VC)	6.14	7.82	42.604	231
GS + RH + VC	6.12	9.20	35.023	213
After				
Garden Soil (GS)	4.52	3.23	17.700	141
GS + Rice Hull (RH)	5.34	5.07	92.171	115
GS + Vermicompost (VC)	4.89	4.31	92.613	297
GS + RH + VC	4.85	5.50	75.396	318

TABLE 2. Nutrient content of bio-organic treatments

Bio-organic nutrients	Nutrient contents					
	% N	% P	% K	% Ca	% Mg	Cu (ppm)
FFJ	0.08	2.50	0.08	0.190	0.047	BD
Seaweed tea	0.08	0.80	0.17	0.280	0.092	BD
Vermi tea	0.02	0.50	0.02	0.009	0.015	BD

BD - Below Detection

Source: Nutrient Analysis Result, SPAL, Central Mindanao University, Musuan, Bukidnon



Error bar represents the standard error of the mean (n=3)

FIGURE 1. Survival rate (%) of stevia at 15 DAT as influenced by growing media applied with bio-organic nutrients

In terms of the interaction effects of the growing media and bio-organic nutrients, results indicated that stevia plants grown in garden soil alone without application of bio-organic fertilizers had the least survival rate of 85% which significantly differed to all plants with different growing media (Table 3). These effects may be credited to the presence of beneficial microorganisms in composted residues and fermented products that gave good physical and biological conditions to the soil, increased aerial and root biomass and survival rate. Plants fertilized with minimal quantities of compost (about 10-20%) had low outputs compared to those added with high doses of vermicompost which significantly increased survival rate of tomato plants (Lazcano et al. 2009). Studies also showed that application of vermicompost or its combination with bio-organic nutrients had better effects on growth parameters of some plants and availability of nutrients present in the soil (Umesha et al. 2011). Moreover, organic fertilizers from various compost products also increased the survival rate of *M. oleifera* plants. Because excessive amounts of nutrients have a negative impact on plant survival, the effectiveness of composts is attributable to their average mineral content (Christophe et al. 2019).

PLANT HEIGHT

As shown in Figure 2, the height of stevia plants was significantly influenced by the growing media while the bio-organic nutrients are not significant ($p \leq 0.05$) throughout the observation period. It was also noticed that the interaction effects of the two (2) independent factors were comparable.

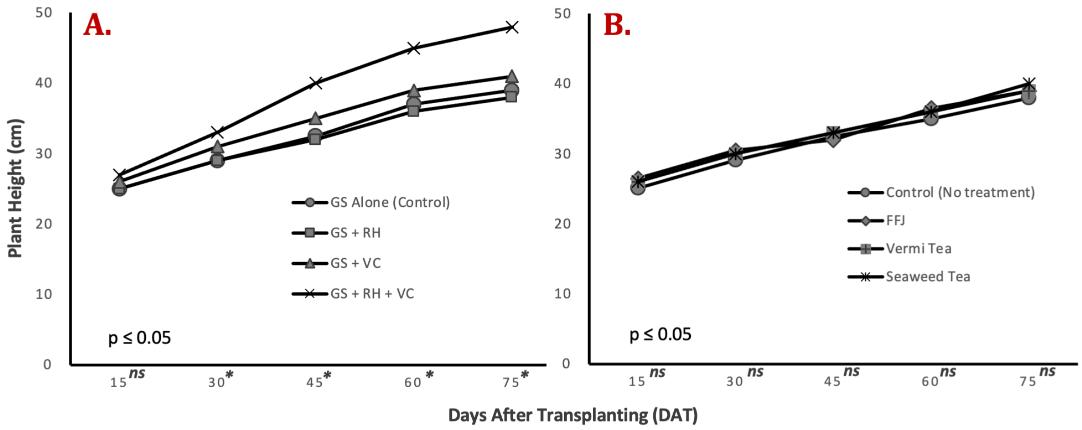
At 15 DAT, stevia plants had more or less similar height regardless of growing media and bio-organic nutrient treatments used. Stevia plants grown in GS+RH+VC consistently exhibited taller plant height compared to other growing media composition from 30 DAT onwards. Stevia plants grown in GS+RH were of shortest height but did not differ from those raised in Garden soil alone. Similar trends in heights of plants were observed at 45, 60 and 75 DAT.

The significant effects on height of stevia plants grown in media mixture GS+RH+VC may be due to its good characteristics of media mixture favorable for the growth of plants. Garden soil (GS) provided the reservoir for nutrients and holds the plants properly in place (Parthasarathi, Balamurugan & Ranganathan 2008). The rice hulls provided also proper aeration and hold moisture

TABLE 3. Interaction effects of growing media and bio-organic nutrients on stevia's survival rate (%) at 15 DAT and number of leaves at 30 and 45 DAT

Treatment combination		Parameter		
Growing media	Bio-organic nutrients	Survival rate (%) at 15 DAT	Number of leaves at 30 DAT	Number of leaves at 45 DAT
GS	Control	85.00 b	20.90 c	29.50 c
	FFJ	97.50 a	42.08 c	61.30 b
	VT	100.00 a	44.73 c	104.58 a
	ST	100.00 a	51.43 b	78.13 b
GS + RH	Control	97.50 a	22.10 c	31.08 c
	FFJ	100.00 a	36.65 c	57.70 c
	VT	100.00 a	31.28 c	79.03 b
	ST	100.00 a	33.60 c	62.28 c
GS + VC	Control	100.00 a	21.73 c	28.10 c
	FFJ	100.00 a	45.73 c	70.63 c
	VT	100.00 a	72.90 a	116.10 a
	ST	100.00 a	85.70 a	97.33 a
GS + RH + VC	Control	100.00 a	21.65 c	31.43 c
	FFJ	100.00 a	50.93 b	75.15 b
	VT	100.00 a	69.15 a	74.88 b
	ST	100.00 a	67.70 a	104.83 a
CV%		2.33	8.32	9.66

Values with different letter superscripts in a column are significantly different ($p \leq 0.05$) based on Tukey's HSD test



*values in a column are significantly different ($p \leq 0.05$) based on Tukey's HSD test; ^{ns} denotes not significant

FIGURE 2. Plant height (cm) of stevia as influenced by growing media (A) and bio-organic nutrients (B) at different days after transplanting

while the vermicast contains micronutrients, hormones and microbial population that protect plants against insect pest and diseases resulting in the best growth of stevia plants (Padhiyar et al. 2017) as evidence in the development of taller plants.

The application of bio-organic nutrients did not affect the height of stevia plants probably be due to lower nutrient contents based on concentration. It was also observed that bio-organic nutrients relatively induced plant height particularly at 60 and 75 DAT only. This may indicate that bio-organic nutrients contain other micronutrients or alkaloids that may possibly be less effective on stevia plants at early stages of growth but may influence growth after two months from transplanting. The delayed effects of bio-organic nutrients on growth of stevia plant can be linked to the levels of elements that require cumulative amounts to become effective. According to Alkorta et al. (1998), fermented fruit waste contains high nutrient levels and microbial diversity that helps supply nutrients to crops which improved the photosynthetic activity leading to an increased height of stevia plants.

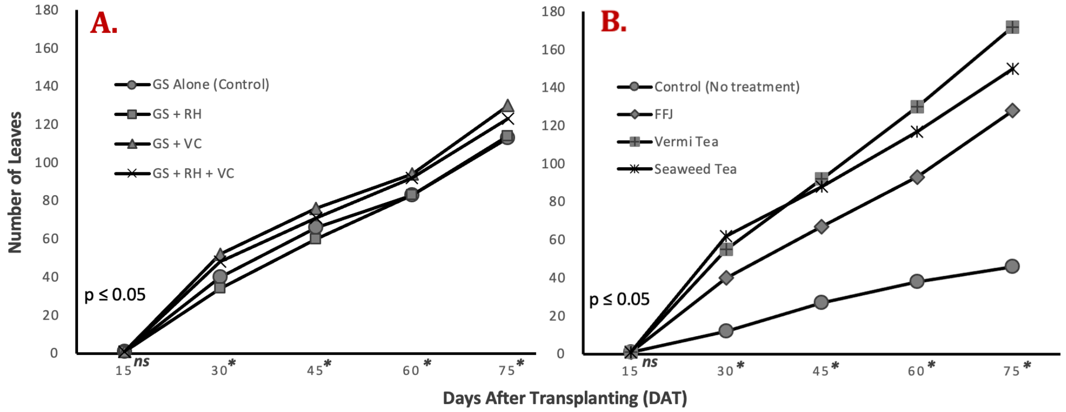
LEAF COUNT PER PLANT

The number of leaves per plant of stevia was counted at various periods (15, 30, 45, 60, and 75 DAT). Statistical analysis ($p \leq 0.05$) showed that the number of leaves developed per plant was significantly affected by growing media and application of bio-organic nutrients throughout the observation period. Specifically, a significant interaction effect on both factors was observed at 30 and 45 DAT (Table 3).

At 30, 45, 60, and 75 DAT (Figure 3), the results showed that stevia plants grown in GS+RH+VC and GS+VC mixtures has developed more leaves compared to other growing media composition. On the same periods, stevia plants applied with Vermi tea significantly developed more leaves per plant compared to other bio-nutrient application with an exception at 30 DAT where Seaweed tea is comparable with Vermi tea.

In terms of the interaction effects of growing media and bio-organic nutrients on the development of stevia leaves, results showed significant results among treatment combinations as reflected in Table 3. Those plants grown in GS+VC which were subsequently applied with Seaweed tea obtained the highest numerical value of 85.70 in terms of the number of leaves developed at 30 DAT. At 45 DAT, plants applied with Vermi tea which were grown under GS+VC had the highest number of leaves developed at 116.10.

The products of vermicomposting have been proven to condition the soil making it favorable for plant growth and development. Vermicast contains millions of beneficial microorganisms that help breakdown organic matter into nutrient structures that are readily accessible to plants (Arancon et al. 2007) that improves structure of the soil, thereby increasing the nutrient retention and soil biological activity (Ghosh et al. 2002). The significant effects of growing media were due to its vermicast component which provided an ample supply of food and energy (Parthasarathi, Balamurugan & Ranganathan 2008) necessary in the development of plant morphology specifically on the development of leaves and leaf area. Composted rice hulls lessen the assimilation of heavy metals to prevent toxicity.



*values in a column are significantly different ($p \leq 0.05$) based on Tukey's HSD test; ^{ns} denotes not significant

FIGURE 3. Number of leaves of stevia at 30, 45, 60, and 75 DAT in response to growing media (A) and bio-organic nutrients (B)

On the one hand, phytohormones, which encourage plant growth, may be the cause of the highly substantial effects of Vermi tea as a bio-organic nutrient (Nogales, Cifuentes & Beníte 2005). The findings of Bess in 2000 that different kinds of Vermi tea have substantial effects on both physiological and biochemical aspects of crops are supported by the results. Additionally, a variety of nutrients included in FFJ and seaweed tea are necessary for the formation of chlorophyll, which keeps plants healthy (Ranch 2018). Alkorta's reports in 1998 which claim that applying any bio-organic nutrients to plants grown on media mixed with vermicast or vermicompost is more effective for better plant growth and development because it provides a long-term nutrient source in comparison to those without application at all, may provide support for the findings.

NUMBER OF SHOOTS

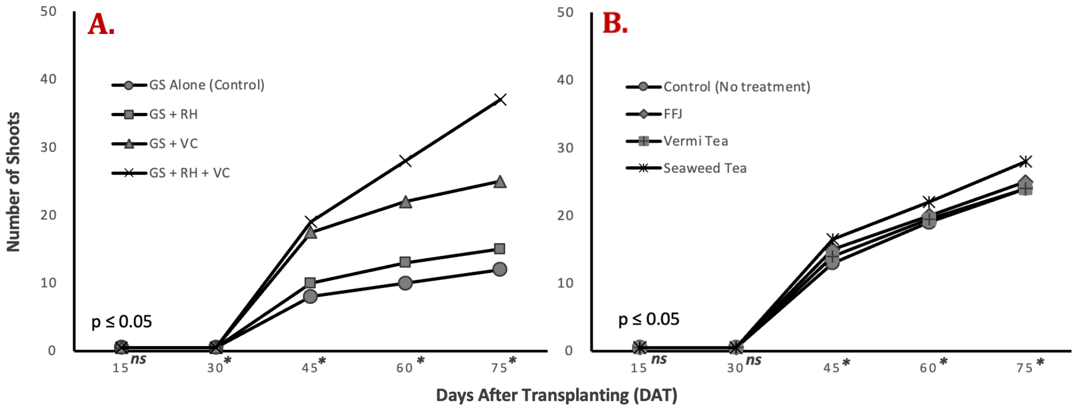
Figure 4 illustrates the number of shoots of stevia plants at 45, 60 and 75 DAT in response to growing media and bio-organic nutrients. Statistical analysis ($p \leq 0.05$) showed that the growing media as well as the bio-organic-nutrients significantly influence the number of shoots of stevia while the interaction of both factors failed to influence this parameter. Results further showed that growing media highly influenced the shoot development of stevia at all observation periods. Stevia plants grown in GS+RH+VC had the greatest number of shoots which significantly differed from the rest of the treatments as observed at 45, 60 and 75 DAT.

Humic acids, and micro- and macronutrients found in vermicompost, composted rice hulls, and garden soil may all be contributing factors to the accelerated development of stevia shoots (Arancon et al. 2007; Valdez-Perez et al. 2011). It is also supported by similar studies that vermicompost and conventional compost retains nutrients for a long time. Studies of Singh et al. (2011) in French bean (*Phaseolus vulgaris*) showed that application of vermicompost or composted bio-organic products lead to early development of shoots and dry biomass. According to reports, this works as a 'wonder growth stimulator and protector' of plants against pests and illnesses.

Application of bio-organic nutrients at 45, 60, and 75 DAT showed that seaweed tea developed the most number of shoots but did not differ significantly from those applied with Vermi tea and FFJ. However, it differed significantly from the control. Results of the present study conform to the findings of Zagro Corporation (2010), who reported that application of seaweed concentrate, produced a greater number of shoots since it contains hormones that enhance the formation of shoots; however, higher dosage can decrease vegetative growth (Ruck 2006). By boosting the available sources of macro and micronutrients through natural processes, bio-organic nutrients, as an organic agricultural input, stimulates growth of plants and the formation of its branches (Vessey 2003).

PERCENT INCIDENCE OF INSECT PESTS AND DISEASES

It was observed that stevia plants were not infested by insect pests nor infected ($p \leq 0.05$) with any plant disease from planting to harvest. Stevia leaves contain substances that act as natural defense against pests like aphids and other bugs. According to Mengesha, Geja and Damtew (2014), stevia plants have been found to have insect repelling tendencies. Likewise, growth media amended with vermicast are more resistant to soil borne diseases since they contain microbial population as protector from pests and diseases.



*values in a column are significantly different ($p \leq 0.05$) based on Tukey's HSD test; ^{ns}denotes not significant

FIGURE 4. Number of shoots of stevia in response to growing media (A) and bio-organic nutrients (B) at 45, 60, and 75 DAT

This study supports de Vasconcelos and Chaves' (2019) results that seaweed extract increases agricultural yields and strengthens plants' tolerance to temperature stress and disease occurrence. The nutrients in the growing mediums encourage the absorption of mineral components from the soil, increase plant resilience to various stresses, encourage rapid development, and ward against pests and diseases.

FRESH WEIGHT OF STEVIA

Fresh weight of experimental plants after harvest was highly affected by growing media and application of bio-organic nutrients (Table 4). Stevia plants grown in GS+RH+VC exhibited higher fresh weight compared to other growing media. In terms of bio-organic nutrients, those plants applied with Vermi tea gained higher fresh weight than other treatments. In terms of the interaction effects of the two factors, statistical analysis ($p \leq 0.05$) showed no significant difference among treatment combinations.

A significant increase on fresh weights of stevia was observed which is in agreement with the synergistic effects of the components of growing media which improved the physical and nutrient condition of soil suitable to plant growth and development resulting in high fresh weight yield of the plants. Studies of Padhiyar et al. (2017) showed that fresh weights of harvested stevia were in positive correlation with the number of roots, its length and total fresh biomass yield. This report indicates that good physical and biological conditions at root zone enhance growth and crop yield (Padhiyar et al. 2017).

The findings of Chen, Wu and Young (2007), who proved that the use of bio-fertilizers is a restorative substitute for chemical fertilizers in giving nutrients to crops and maintaining soil health for sustainability, are also the best evidence for the outcomes. The outcomes of the research of Das et al. (2007) as previously mentioned, showed that the yield of stevia plants increased as a result of their capacity to fix atmospheric nitrogen (symbiotic fixation), convert innate nutrients in the soil (e.g., P, Zn, Cu, Fe and S) from fixed or non-usable to usable forms as well as decomposed organic wastes through biological processes. Moreover, the present result on the application of bio-organic nutrients is consistent with the study of Padhiyar et al. (2017).

DRY WEIGHT OF STEVIA

Table 4 also presents the average weight (dry) of stevia plants in response to growing media and bio-organic nutrients. Statistical analysis ($p \leq 0.05$) showed that the dry weights of stevia plants were highly influenced by the growing media and bio-organic nutrients at second harvest. On the other hand, no significant interaction effects on both factors were observed on the weight (dry) of air-dried leaves of stevia. Similar to fresh weight, the interaction effects also failed to show any significant difference among treatment combinations.

TABLE 4. Fresh weight, dry weight, herbage yield and visual quality rating of Stevia in response to growing media and bio-organic nutrients

Treatment	Fresh weight (g)		Dry weight (g)		Herbage yield (g)		Visual quality rating	
Growing Media (A)							*ns*	
Garden Soil (GS)	77.75	d	17.85	d	2,097.50	d	4.25	
GS + Rice Hull (RH)	152	c	36.82	c	4,058.12	c	4.75	
GS + Vermicast (VC)	193.38	b	48.99	b	5,465.62	b	4.88	
GS + RH + VC	297.81	a	78.61	a	7,231.25	a	4.88	
Bio-Organic Nutrient (B)								
Control	177.94	c	44.57	b	3,847.50	c	4.25	b
FFJ	146.88	b	35.83	c	5,008.75	b	4.75	b
Vermi tea	218.69	a	56.49	a	5,486.88	a	4.88	a
Seaweed tea	177.44	b	45.38	b	4,509.38	b	5.00	a
Interaction Effects (AXB)	*ns*		*ns*		*ns*		*ns*	

Values with different letter superscripts in a column are significantly different ($p \leq 0.05$) based on Tukey's HSD test; ns denotes not significant

Dry weight of plants grown on GS+RH+VC produced higher dry weight compared to other media composition while garden soil alone significantly produced the least dry weight. For bio-organic nutrients, stevia plants applied with Vermi tea relatively obtained the greater dry weight among treatments. In addition, those applied with FFJ significantly produced lesser dry weight of stevia leaves.

Due to the presence of various bacterial strains that can fix nitrogen and dissolve the phosphorus and potassium necessary for stevia's growth and development, stevia leaves' dry weight significantly increased in response to the combination of vermicast, composted rice hulls, and garden soil. This resulted in high dry weight. In comparison to plants cultivated in soil without vermicompost, vermicompost alone or in combination with other media components significantly affected the dry weight of stevia. According to Zaman et al. (2015), applying VC at all stages improved leaf dry weight at harvest in acid soil by up to 119-335%.

Results on high dry matter weight of stevia applied with bio-organic nutrients maybe due to its ability to improve plant growth parameters resulting from nutrient availability thus improved root growth and hormone production by microorganisms. These processes in return increased production of carbohydrate materials and organic structures which consequently increased dry weight. Weaver (1972) found that hormone production by these microbial populations has major roles to increase stevia leaf area, fresh and dry weight that leads to increased herbage yield.

HERBAGE YIELD (G) OF STEVIA

Herbage yield of stevia is presented in Table 4. Statistical analysis ($p \leq 0.05$) showed that herbage yield was highly affected by the growing media and application of bio-organic nutrients while no significant interaction effects of both factors were observed. Stevia grown in GS+RH+VC had the highest herbage yield those grown in garden soil alone produced the least herbage yield statistically and numerically. On the other hand, stevia plants applied with Vermi tea exhibited the highest herbage yield among treatments.

Vermicompost is essential for improving soil aeration, texture, and compaction, which leads to better plant water and nutrient uptake from surrounding areas of the root zone (Edwards, Arancon & Greytak 2006). Vermicast application promotes plant growth, germination, root development, and enhanced production of herbage crops, whether used alone or in combination with other organic media. Numerous researches have noted the advantages of bio-organic fertilizers like compost for crop productivity. When combined with organic products, bio-fertilizers, which are microbial in origin and have an efficient role for cell proliferation, are useful for enhancing crop yield, quality, and production, according to Kumar and Upadhyaya (2012).

VISUAL QUALITY RATING OF LEAVES

Table 4 presents the visual quality rating of leaves of stevia as influenced by growing media and bio-organic nutrients. Results of statistics ($p \leq 0.05$) showed that this parameter was not significantly affected by growing media but significantly influenced by the treatment of bio-organic nutrients. Nonetheless, there were no significant interactions between the growing media and bio-organic nutrients employed.

The growing media failed to influence the visual quality rating of the color which ranged from 4.25 to 4.88 described as dark green in color with fully expanded leaves attached on long stalks. For bio-organic nutrients, statistical results showed that stevia plants applied with seaweed tea had a visual quality of 5.00 which is comparable to Stevia plants applied with Vermi tea. These treatments, however differed significantly from those applied with FFJ and the Control with VQR of 4.75 and 4.25, respectively.

The production of chlorophyll by plants generated from fermented extracts is responsible for the stevia plants' characteristically green leaves. Nitrogen, a crucial component required for the creation of chlorophyll that supported plant development and vigor, was given to the plants through the use of growing media with combinations of composted rice husk, garden soil, and vermicompost (Ranch 2018). Likewise, the exposure of stevia plants to external factors such as sunlight, relative humidity, temperature, and rainfall provided with the same cultural management practices such as watering, weed management, and others resulted in chlorophyll formation reflecting the excellent leaf color 'dark green' resulting in good photosynthetic activities, physiological processes, growth, and development (Zagro Corporation 2010).

CONCLUSIONS

Among the different growing media composition, it appeared that the use of garden soil in combination with rice hulls and vermicompost (GS+RH+VC) promotes better and desirable herbage growth, yield and quality of Stevia as manifested in the statistical analysis. These parameters include survival rate, plant height, number of leaves and shoots per plant, fresh and dry weight and herbage yield. Vermi tea, on the other hand, performed well as a bio-organic nutrient for stevia as it enhanced its growth and herbage yield. In terms of the interaction effects, application of Vermi tea on stevia plants grown in garden soil mixed with vermicompost (GS+VC) gained more number of leaves than other treatment combinations.

RECOMMENDATIONS

Based on the findings of the study, it is recommended to delve deeper into the optimal application methods and dosage of Vermi tea, as well as explore potential synergies with other bio-organic nutrients such as vermi drippings and organic manures. Considering the consistent positive impacts of bio-organic nutrients and growing media compositions, it is recommended to explore the scalability and practical application of these findings in larger-scale Stevia cultivation. This may involve field trials and implementation studies to assess the feasibility of integrating these practices into commercial production. Moreover, exploring on the postharvest processing aspect as well as the determining the chemical components of Stevia leaf particularly on stevioside, from Stevia plants applied with bio-organic nutrients is also empirical in advancing sustainable and high-quality Stevia production, addressing the increasing demand for natural sweeteners in a health-conscious market.

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