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Growth Performance of Stevia (*Stevia rebaudiana* Bertoni) as Influenced by Clonal Propagation Methods and Growing Media

Eric Randy R. Politud

Institute of Agriculture, Misamis Oriental State, College of Agriculture and Technology, Claveria, 9004 Misamis Oriental, Philippines
Corresponding author: Eric Randy R. Politud, email: erpolitud@yahoo.com

Abstract

The study was conducted to evaluate the growth performance of Stevia as influenced by clonal propagation methods and growing media at the Nursery House of the City Agriculture Office, Casisang, Malaybalay City, Bukidnon, Philippines from September 2015 to November 2015. The study was carried-out in a 3 x 5 factorial arrangement in Completely Randomized Design (CRD) with three replicates with cutting methods (shoot tip cuttings, nodal cuttings and wedge stem cuttings) as Factor A and growing media (pure garden soil, ½ garden soil + ½ vermicast, pure vermicast, ½ garden soil + ½ sand, and 1/3 garden soil + 1/3 sand + 1/3 vermicast) as Factor B. Results revealed that shoot tip cuttings in combination with 1/3 garden soil + 1/3 sand + 1/3 vermicast in plastic cups two months after planting significantly produced the tallest plants (13.72 cm), highest survival rates (96% for Factor A and 97.33% for Factor B), least incidence of leaf curling and leaf dark spot (both 1.22 ratings for Factor B), and most number of leaves (26.67) and branches (3.25). However, the combination of nodal cuttings and pure garden soil showed very poor growth performance in all parameters evaluated. Thus, propagating Stevia using shoot tip cuttings with 1/3 garden soil + 1/3 sand + 1/3 vermicast media mixture using plastic cups for two months showed the best growth performance, hence, recommended for mass propagation and herbage production.

Keywords: herbage, nodal, shoot, stem, vermicast

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Introduction

Stevia (*Stevia rebaudiana* Bertoni) commonly known as sweet leaf or sugar leaf is a natural and healthy alternative to sugar and artificial sweeteners. It is a widely known perennial shrub of Asteraceae family (Geuns, 2007; Lemus-Mondaca et al., 2012). It is extensively grown in the subtropical regions. Stevia has long been widely used as a sweetener in beverages and to mask the bitter taste of certain herbal medicinal plants in several countries like Brazil, Japan, Paraguay, and others (Parsons & Cuthberston, 2001;), however, it is not well known in the Philippines.

The leaves of Stevia are the sources of stevioside, a natural sweetener and is 250-300 times sweeter than sucrose, heat stable, pH stable, non-fermentable and has no effect on blood sugar (Ghanta et al., 2007; Goyal & Samsher, 2010; Ritu & Nandini, 2016). Hence, Stevia is considered and allowed to be called as sweetener by the Food and Drug Administration (FDA) of the US in 2008. Stevia alleviates hypoglycemia and type 2 diabetes (Soejarto et al., 1983; Ramesh et al*.,* 2006), nourishes pancreas, and thereby helps restore its normal function. It also contains high percentage of phenols that reduce the cardiac and cancer diseases (Dragovi-Uzelac et al., 2010) and flavonoids which have high antioxidant activity (Tadhani et al., 2007; Shukla et al., 2009).

Apart from sweetness, Stevia is imbued with stevioside-related compounds of rebaudioside A and steviol that offer therapeutic benefits like antihypertensive, antidiabetic, anti-inflammatory, anti-tumor, antioxidant, antidiarrheal, diuretic, and immunomodulatory actions. Steviol interacts with the drug transporters, hence proposed as drug modulator (Goyal & Samsher, 2010).

The economic importance, health benefits, and high demand of Stevia in both local and export markets provide great production, income and livelihood opportunities for farmers, hobbyists and households. Moreover, the crop is adapted over a wide range of climatic conditions in the country and apparently been successfully grown abroad (Qui et al., 2000).

At present, little is known about Stevia as well as its propagation, postharvest handling, and processing. There are no protocols on rapid clonal propagation and growing media, particularly in Mindanao or Bukidnon. Thus, the objective of this study was to evaluate the growth performance of Stevia (S*tevia rebaudiana* Bert.) as influenced by methods of clonal propagation and growing media.

Materials and Methods

The study was conducted at the Nursery House of the City Agriculture Office, Casisang, Malaybalay City, Bukidnon for two months starting September 2015 to November 2015. The study utilized Stevia mother plants (*Stevia rebaudiana* Bertoni) and B-net screened nursery structures (9.5 m long x 5.5 m wide x 3 m high) with 50 to 60% shade.

This study was laid-out in a 3 x 5 Factorial Arrangement in Completely Randomized Design (CRD) replicated three times with 20 samples per replication. The three types of cuttings of Stevia served as Factor A and the five combinations of growing media as Factor B are indicated below:

Factor A (Types of Cutting)	**Factor B (Growing Media)**
A_1 - Shoot tip	B_1 - Pure garden soil (control)
A_2 - Nodal	B_2 - ½ Garden soil + ½ Vermicast
A_3 - Wedge stem	B_3 - Pure vermicast
	B_4 - ½ Vermicast +½ Sand
	B_5 - 1/3 Vermicast+1/3 Garden soil + 1/3 Sand

The different treatments and treatment combinations are shown in Table 1. A two-layer 2-ply B-net nursery was established with a height of 3.0 meters and a considerable 50-60% shade in the inside condition. It has a strong support of metal and wooden frames including the door. The ground terrain inside the nursery was elevated and was compartmentalized with cemented boxes for the set-ups. The terrain was rolling; hence, canals were defined along the cemented boxes.

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Table 1. Treatments and treatment combinations.

Types of cutting (factor a)	Growing media (factor b)	Treatment combinations/ Codes	Treatments
Shoot tip (A_1)	▪ Pure garden soil (Control –B_1)	A_1B_1	T_1
	▪ ½ Garden soil + ½ Vermicast (B_2)	A_1B_2	T_2
	▪ Pure vermicast (B_3)	A_1B_3	T_3
	▪ ½ Vermicast + ½ Sand (B_4)	A_1B_4	T_4
	▪ 1/3 Vermicast + 1/3 Garden soil + 1/3 Sand (B_5)	A_1B_5	T_5
Nodal (A_2)	▪ Pure garden soil (Control –B_1)	A_2B_1	T_6
	▪ ½ garden soil + ½ Vermicast (B_2)	A_2B_2	T_7
	▪ Pure vermicast (B_3)	A_2B_3	T_8
	▪ ½ Vermicast + ½ Sand (B_4)	A_2B_4	T_9
	▪ 1/3 Vermicast + 1/3 Garden soil + 1/3 Sand (B_5)	A_2B_5	T_{10}
Wedge stem (A_3)	▪ Pure garden soil (Control –B_1)	A_3B_1	T_{11}
	▪ ½ Garden soil + ½ Vermicast (B_2)	A_3B_2	T_{12}
	▪ Pure vermicast (B_3)	A_3B_3	T_{13}
	▪ ½ Vermicast + ½ Sand (B_4)	A_3B_4	T_{14}
	▪ 1/3 Vermicast + 1/3 Garden soil + 1/3 Sand (B_5)	A_3B_5	T_{15}

The study utilized 10 sq.m. area (10.0 m long and 1.0 m wide) and was enclosed by a screened nursery. The area was divided into 45 small plots of equal sizes (0.31 m x 0.38 m or an area of 0.12 sq.m. each plot) corresponding 15 treatments with an alleyway between plots of 0.076 m. as well as 0.5 m from plots to the nursery wall.

A composite sample of 1.0 kg for each of the growing media was taken and air-dried for chemical analysis. These air-dried samples were then submitted to the City Soil Testing Laboratory, City Agriculture Office, Casisang, Malaybalay City for routine analysis such as organic matter (OM), phosphorus (P), potassium (K), and pH. All growing media were sterilized using hot water treatment prior to polypotting.

Stevia planting materials were purchased from a nursery in Baungon, Bukidnon, and the plastic cups (7 oz) from an agricultural supply at Malaybalay City. The garden soil and sand were procured from the garden area of the City Agriculture Office, Casisang, Malaybalay City, while the vermicast from an agricultural supply in Malaybalay City.

Each growing medium was weighed equally prior to polypotting with the vermicast as the basis for all the growing media combinations for all the pots. For the growing media combinations, a 100% pure garden soil, 1/2 garden soil + 1/2 vermicast, 100% vermicast, 1/2 vermicast + 1/2 sand and 1/3 vermicast + 1/3 garden soil + 1/3 sand were allocated to various treatment samples. Twenty plastic cups filled with media mixtures were placed according to their respective lay-out per treatment per replication.

Three nodes shoot tip cuttings, one node nodal cutting and one inch wedge stem cutting for each sample were the types of cuttings prepared. Nodal and wedge stem cuttings were taken from the medium to matured stems of Stevia mother plants. All the cuttings were dipped in tap water prior to planting. One cutting was planted per pot.

A total of 900 cuttings were planted for the entire study with one cutting per pot. The potted plastic cups were watered using the sprinkler before planting to prevent the cuttings from washing-out if sprinkling is done after planting. Labeling was done according to respective treatments. A 10 m long x 1.25 m wide transparent polyethylene cellophane was established 0.5 m above the experimental set-up after planting the cuttings to the pots to prevent the young cuttings from direct heat of the sun and heavy rains.

Water was applied to the cuttings 2-3 times a day or as needed. The soil was kept moist all the time, but not water soaked, to prevent the cuttings from wilting. The growing media were cultivated from time to time where the cuttings were grown to facilitate proper aeration for the cuttings to produce roots. Diseases attacking the plants were not controlled, but were being observed and recorded.

Among the data gathered were the average plant height, survival rate, incidence of leaf curling and leaf dark spot, and the number of branches and leaves per plant. The analysis of variance (ANOVA) using factorial in Completely Randomized Design (CRD) was carried out to

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solve for the level of significance. The Tukey's test was used to compare significant differences among the treatment means.

Results and Discussion

Average plant height (cm)

Statistical analysis revealed that heights of stevia cuttings 60 days after planting were highly influenced by the propagation methods (Factor A). Similarly, the respective heights of the plants in Factor B were affected by the growing media used. However, no significant interaction effect was observed between the two factors (Table 2).

Table 2. Average plant height (cm) of Stevia (*Stevia rebaudiana* Bert.) cuttings at 60 days after planting (DAP) in response to different growing media.

Growing media (B)	Propagation methods (A)			Mean (B)*
	Shoot tip cutting	Nodal cutting	Wedge stem cutting	
Pure garden soil	11.43	3.73	3.90	6.35[b]
½ Garden soil + ½ Vermicast	16.23	4.97	5.23	8.81[a]
Pure vermicast	13.81	4.33	4.43	7.52[ab]
½ Sand + ½ Vermicast	12.48	4.57	4.80	7.28[ab]
1/3 Garden soil + 1/3 Vermicast + 1/3 Sand	14.67	4.93	5.83	8.48[ab]
Mean (A)**	13.72[a]	4.51[b]	4.84[b]	
F-test: A**, B*, A x B [ns]				
CV: 20.53%				

Means within the same column and row followed by common letters are not significantly different at 5% level of significance based on Tukey's Test.
** - highly significant,* - significant, ns - non-significant

The types of cuttings in propagating Stevia produced different heights at the end of the study. Shoot tip cuttings had the tallest plants with an average height of 13.72 cm which were significantly different from those propagated using wedge stem and nodal cuttings with 4.84 cm and 4.51 cm, respectively (Figure 1). Since the shoot tip cuttings used at the start of the study had an average height of 5 cm containing 3 nodes from the tip while the wedge stem and nodal cuttings were just bulging the bud eyes from the stems, it is therefore expected that those grown using shoot tips had unprecedented leap of the heights compared to the latter two months after the study started. Shoot tip cuttings bind to the idea that growth is fast due to the controlling power of auxin for apical dominance (Hartmann & Kester, 2013).

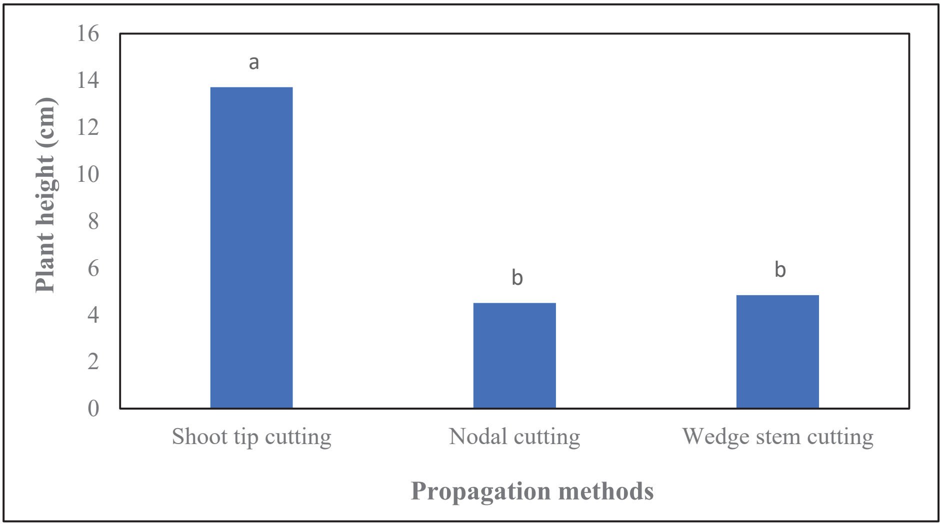

Figure 1. **Average plant height (cm) of Stevia at 60 days after planting as affected by propagation methods.**

According to Lab (2012), auxin induces shoot apical dominance. The axillary buds are inhibited by auxin, as a high concentration of auxin directly stimulates ethylene synthesis in axillary buds, causing inhibition of their growth and potentiation of apical dominance. When the apex of the plant is removed, the inhibitory effect is removed, and the growth of lateral buds is enhanced. Moreover, auxin is sent to the part of the plant facing away from the light, where it promotes cell elongation. If there is no cutting in the apical part or the shoot tip, the growth would be faster since auxin is polar, hence, more of its concentration focuses on the apex part.

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The growth and proliferation of the cuttings have something to do with the various growing media used based on the independent interaction effects of each factor. For the various growing media, it showed also that cuttings grown in ½ garden soil + ½ vermicast had the tallest plant with 8.81 cm upon the termination of the study (Figure 2). It was, however, not significantly different from those grown in 1/3 garden soil + 1/3 vermicast + 1/3 sand and 100% pure vermicast with 8.48 cm and 7.53 cm plant height, respectively, but were statistically far from those grown in pure garden soil with 6.35 cm only. Based from the soil analysis of the growing media and the kinds of the media, nutrients are contributory to the growth performance of the cuttings especially plant height. The three macronutrients such as nitrogen, phosphorus, and potassium have special roles on the growth and development of the plants which were contained in four organic growing media except with few amounts in pure garden soil alone (Bautista, 1994; Hartmann & Kester, 2013).

Survival rate

Statistical analysis showed no significant difference observed regardless of the types of cuttings (Factor A) and the growing media (Factor B) used. No interaction effect was likewise observed between the two factors (Table 3). However, the combination of shoot tip cuttings with 1/3 garden soil + 1/3 vermicast + 1/3 sand as growing media obtained the highest survival rate with 96% for the former and 97.33% for the latter, respectively, although their differences were not that significantly different from the rest of the treatment combinations.

Stevia plants using nodal cuttings in Factor A obtained the lowest survival rate with 92.07%, while those grown in pure garden soil in Factor B had 92.22% survival rate. According to Kassahun et al. (2013), top cuttings or shoot tip cuttings with three nodes among Stevia plants demonstrated significantly higher values of survival rate (82.5%), but are not that far from cuttings taken at the bottom.

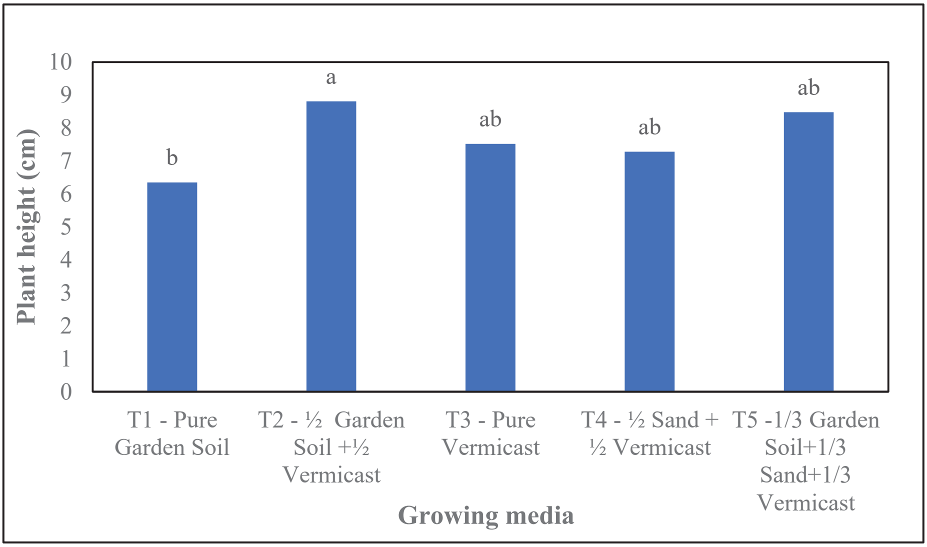

Figure 2. **Average plant height (cm) of Stevia at 60 days after planting as affected by growing media.**

Table 3. **Survival rate (%) of Stevia cuttings in response to the different (A) propagation methods and (B) growing media.**

Treatments	Survival rate (%)
Propagation methods (A)	
Shoot tip cutting	96.00
Nodal cutting	92.07
Wedge stem cutting	93.67
Growing media (B)	
Garden soil	92.22
½ Garden soil + ½ Vermicast	96.11
Vermicast	92.78
½ Sand + ½ Vermicast	91.11
1/3 Garden soil + 1/3 Vermicast + 1/3 Sand	97.33
F-test: A, B, A x B	ns
CV (%)	6.20

ns – non-significant

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Consistently in this study, shoot tip cuttings also obtained the highest survival rate at 96% compared to nodal and wedge stem which had only 92.07% and 93.67%, respectively. Considering that the whole set-ups were placed under screened house or B-net nursery, survival rate had been high for all the treatments.

Leaf curling and leaf dark spot incidence

No significant differences were observed regardless of the types of cuttings and growing media as well as their respective interaction effects (Table 4). Leaf curling occurred in plants especially Stevia in the younger stage due to deficiency in calcium in the growing media, thus making the leaves to have downward position (Ceunen & Geuns, 2010). The highest incidence of leaf curling was observed in propagated cuttings (Factor B) grown in garden soil with 2.0, while the lowest was on cuttings grown in media with 1/3 garden soil + 1/3 vermicast + 1/3 sand with 1.22 incidence per plot. However, their differences were not that significant regardless of the growing media used.

Table 4. Leaf curling and leaf dark spot incidence in Stevia cuttings in response to the different (A) propagation methods and (B) growing media.

Treatments	Leaf curling incidence	Leaf dark spot incidence
Propagation methods (A)		
Shoot tip cutting	1.47	1.73
Nodal cutting	1.80	1.53
Wedge stem cutting	1.40	1.53
Growing media (B)		
Garden soil	2.00	1.89
½ Garden soil + ½ Vermicast	1.56	1.67
Vermicast	1.44	1.67
½ Sand + ½ Vermicast	1.56	1.56
1/3 Garden soil + 1/3 Vermicast + 1/3 Sand	1.22	1.22
F-test: A, B, A x B	ns	ns
CV (%)	14.55	19.46

ns – non-significant

For the propagation methods (Factor A), it was revealed that Stevia cuttings using nodes obtained the highest incidence of leaf curling per plot with 1.80, while the lowest was with those propagated using wedge stems with 1.40 occurrences. Their differences, however, were found not significantly different from each other.

For leaf dark spots, it showed that the lower incidence was observed on cuttings propagated using nodes and wedge stems (both 1.53) in Factor A, with a bit higher incidence on those propagated using shoot tips with 1.73. However, their differences were said to be not significantly far from each other. Likewise, it was revealed in Factor B that stevia cuttings grown in pure garden soil had the most number of leaf dark spots with 1.89, while the least with those grown in 1/3 garden soil + 1/3 vermicast + 1/3 sand with 1.22 plants infected per plot. According to Cuenen (2010), leaf dark spots among Stevia plants are due to lack of calcium resulting to chlorosis and necrosis of leaves.

The incidence of both leaf curling and leaf dark spots in Stevia plants only ranged from 1 to 2 plants per plot with a total of 20 plants in a plot (or only 5 to 10% of the plants in the plot). The degree of damage was just slight and insignificant since only few leaves in selected plants were infected with the symptoms.

Average number of branches per rooted cutting

Propagation methods (Factor A) and growing media (Factor B) highly influenced the number of branches per rooted cutting. No interaction effect was found between these factors (Table 5). Stevia grown using shoot tip cuttings obtained most number of branches per plant with 2.69 and was significantly different from those using wedge stem and nodal cuttings with 1.17 and 0.99, respectively (Figure 3). Propagating Stevia using wedge stem cuttings was not statistically different from those using nodal cuttings.

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Table 5. Average number of branches per rooted cutting of Stevia cuttings in response to growing media.

Growing media (b)	Propagation methods (a)			Mean (B)*
	Shoot tip cutting	Nodal cutting	Wedge stem cutting	
Pure garden soil	2.43	0.23	0.50	0.99[b]
½ Garden soil + ½ Vermicast	2.63	1.17	1.40	1.73[a]
Pure vermicast	2.71	1.00	1.17	1.62[a]
½ Sand + ½ Vermicast	2.61	1.27	1.20	1.69[a]
1/3 Garden soil + 1/3 Vermicast + 1/3 Sand	3.25	1.30	1.57	2.04[a]
Mean (A)**	2.69[a]	0.99[b]	1.17[b]	
F-test: A**, B*, A x B[ns]				
CV: 26.86%				

Means within the same column and row followed by a common letter are not significantly different at 5% level of significance based on Tukey's Test.
* - highly significant, ns – non-significant

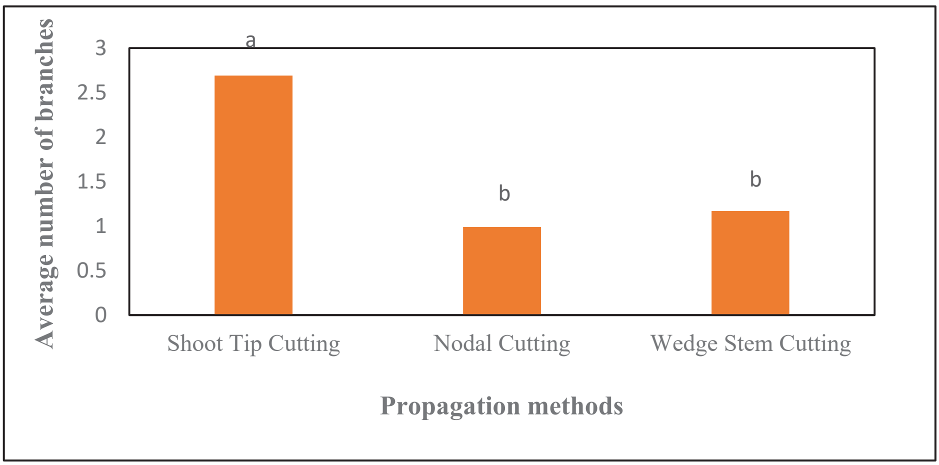

Figure 3. Average number of branches per rooted cutting of Stevia as affected by propagation methods.

Gvasaliye et al. (1990) also reported that the rooting of cuttings was best in cuttings taken from side shoots and from tops of the main shoot in honey grass. Cuttings from the top part of the main stem of

Stevia generally gave the best result (Tirtoboma, 1988). Top cuttings with three nodes demonstrated significantly higher values in the number of branches per seedling (Kassahun et al., 2013). According to Hartmann and Kester (2013), apical dominance is apparent among shoot tips with high concentration of auxin, thus, promoting growth faster. Shoot tips were already defined having 3 nodes from the tip which were used in the study as compared to nodal and wedge stem cuttings where the buds were still bulging and whose stem nodes have yet to be defined.

Similarly, Stevia cuttings grown in 1/3 garden soil + 1/3 vermicast + 1/3 sand had the most average number of branches per plant with 2.04. It was, however, not significantly different from those grown in ½ garden soil + ½ vermicast, ½ sand + ½ vermicast and pure vermicast with 1.73, 1.69, and 1.62 branches, respectively (Figure 4). Those grown in pure garden soil only obtained the least with 0.99 branch per plant.

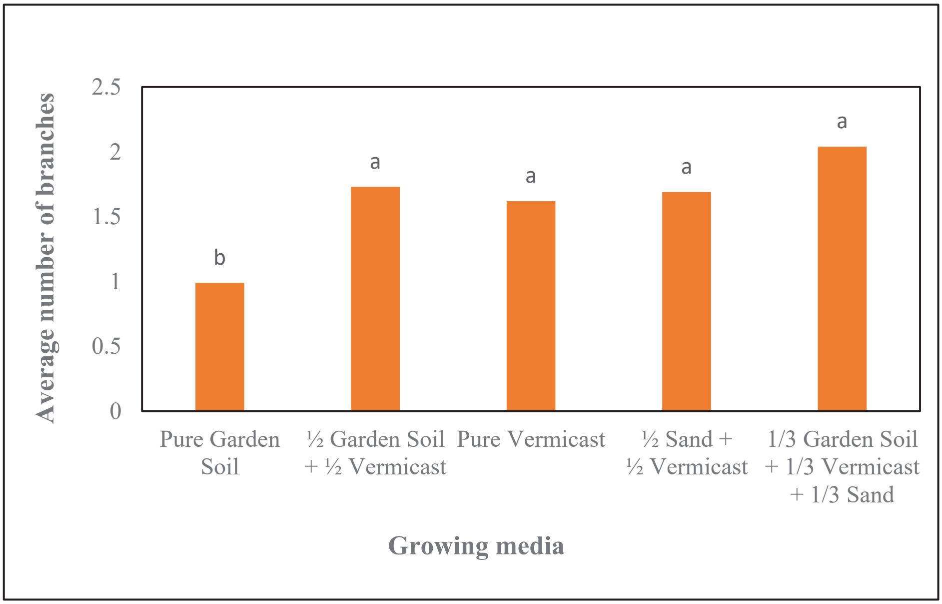

Figure 4. Average number of branches per rooted cutting of Stevia as affected by growing media.

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Hartmann and Kester (2013) emphasized the roles of nutrients such as nitrogen, phosphorus, and potassium in the cell division, differentiation, and elongation as well as nutrient uptake and transport to different growth regions in plants, thus, giving rise to the development of many nodal points and branches. Top cuttings with three nodes demonstrated significantly higher values in the number of branches/seedling (7), number of leaves/branch (15), and number of leaves/seedling (56) with the lowest values of these parameters recorded for bottom cuttings (Kassahun et al., 2013).

Average number of leaves per plant

Propagation methods and growing media greatly influenced the production of leaves of Stevia two months after planting with no interaction effect found between the two factors (Table 6). Production of leaves conforms to the production of branches as mentioned above on propagation methods and growing media as well as their respective interaction effects. The number of branches likewise determines the numbers of leaves since nodes are borne to every branch.

Table 6. Average number of leaves per plant of Stevia cuttings in response to growing media.

Growing media (B)	Propagation methods (A)			Mean (B)*
	Shoot tip cutting	Nodal cutting	Wedge stem cutting	
Pure garden soil	18.00	10.67	12.00	13.56^{d}
½ Garden soil + ½ Vermicast	22.67	17.33	17.67	19.22^{ab}
Pure vermicast	21.67	14.33	14.67	16.89^{c}
½ Sand + ½ Vermicast	21.00	15.33	16.00	17.44^{bc}
1/3 Garden soil + 1/3 Vermicast + 1/3 Sand	26.67	16.00	19.00	20.56^{a}
Mean (A)**	22.00^{a}	14.73^{b}	15.87^{b}	
F-test: A**, B*, A x B^{ns}				
CV: 8.84%				

Means within the same columns and rows followed by common letters are not significantly different at 5% level of significance based on Tukey's Test.
** - highly significant, ns - non-significant

On propagation methods, it showed that Stevia propagated through shoot tip cuttings obtained the most number of leaves with 22 and was significantly different from those grown using nodal and wedge stem cuttings with 15.87 and 14.73, respectively (Figure 5). It is also evident that as more branches were produced using shoot tip cuttings, more leaves were likewise being borne because many nodes were also produced regardless of the sizes of the leaves. Faster growth was also observed among shoot tip cuttings as compared to nodal and wedge stems. Cuttings from the top part of the main stem of stevia generally gave the best result (Tirtoboma, 1988).

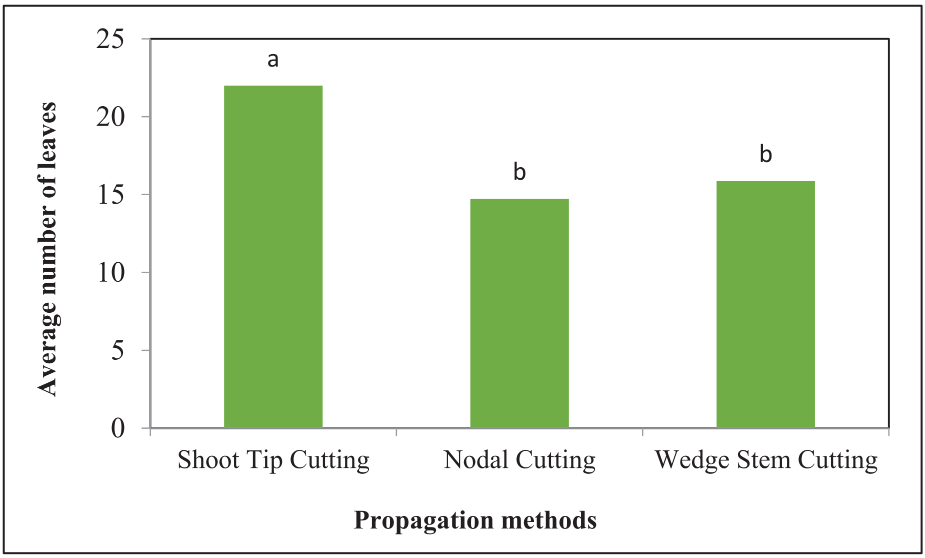

Figure 5. Average number of leaves per plant of Stevia as affected by propagation methods.

Results on growing media revealed that the combination of 1/3 garden soil + 1/3 vermicast + 1/3 sand produced the most number of leaves per plant with 20.56 regardless of the independent interaction effects of the two factors (Figure 6). It was, however, not significantly different from those grown using ½ garden soil + ½ vermicast with 19.22, but is statistically different from the rest of the treatment

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combinations. Those grown using pure garden soil only obtained 13.56 leaves per plot. Stevia grows well in sandy loam soils with enough supply of water during the month of October as exhibited in the balanced media combinations of 1/3 garden soil + 1/3 sand + 1/3 vermicast. Stevia prefers acidic to neutral soil with a pH range of 6.5-7.5 for its best growth. Saline soils should be avoided as Stevia plant is susceptible to water logged conditions, hence, Stevia will hardly grow in saline soils (Todd, 2010).

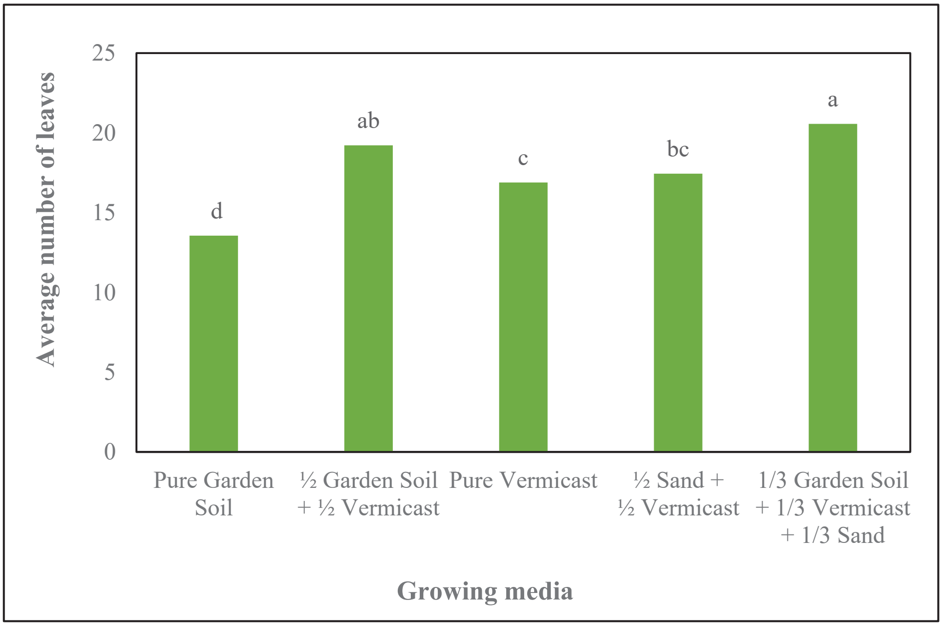

Figure 6. **Average number of leaves per plant of Stevia as affected by growing media.**

Conclusion and Recommendations

Propagating Stevia shoot tip cuttings using 1/3 garden soil + 1/3 sand + 1/3 vermicast in plastic pots showed the best growth performance among the rest of the treatments compared. Hence, it is recommended for mass production and herbage yield.

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