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Abstract

Fertilizers are recognized as limiting factors for crop growth in Rwanda. The choice of fertilizers to be used for a certain crop in certain soil is crucial for production. The experiment was conducted in Rubona and Ntendezi stations of Rwanda Agriculture and Animal resources Development Board (RAB) during seasons 2021A and 2021B respectively. The objectives were to develop guidelines for stevia production in Rwanda and increase production through promotion and utilization of good quality fertilizers in stevia production. The treatments included four types of organic manures (cow dung, poultry manure, compost and pig manure), three inorganic fertilizers (NPK, DAP and urea) and control or check (without fertilizer Plant sampling was done once a week to measure plant height, number of tillers, internodes and to measure yield at harvest. . The results revealed a significant interaction between treatments and plant height. Poultry manure played more dominant role in increasing plant height (44.51m), cow dung was the second (43.50m) while control treatment showed minimum contribution in increasing number of stevia height (39.76m). Poultry manure also played dominant role in increasing number of tillers (22.76), urea was the second (21.0) while control treatment showed minimum contribution in increasing number of stevia tillers (16.8). The yield of harvested fresh leaves of stevia that resulted from poultry manure has shown at least height yield in comparison with other treatments.

Keywords: *Fertilizers, Types of fertilizers, Stevia rebaudiana, production*

1.1 Introduction

Stevia (*Stevia rebaudiana Bertoni*) is an herbaceous perennial plant of the Asteraceae family, originating from the north-east of Paraguay, where it grows wild in sandy soils [1]. The genus Stevia contains about 154 species; six species are widely utilized that are *Stevia eupatoria*, *Stevia ovata*, *Stevia plummerae*, *Stevia salicifollia*, *Stevia serrata* and *Stevia rebaundiana*. From these, *Stevia rebaundiana* is the one with significant sweetening properties. The property of the species that called attention to this plant was the intense sweet taste of the leaves and aqueous extracts. The leaves of stevia contain sweetening compounds (glycosides) namely Stevioside, Rebaudioside A, Rebaudioside B and Rebaudioside C and six other compounds which have insulin balancing properties. The sweeteners impart 250 times sweetness than table sugar and 300 times more than sucrose. The glycosides are extracted from the Stevia leaf as all-natural zero caloric sweeteners; hence, stevia has been named as calorie free bio-sweetener of high quality with non-fermentable, non-discoloring, maintain heat stability at100^{o}c and features a lengthy shelf life attribute [2]. Dry leaves are the economic part of the stevia plant [2], with a high concentration of steviol glycosides, possible substitutes of synthetic sweeteners [3] which are many times sweeter than sugarcane and sugar beet but importantly without any calories [4].

The main steviol glycosides in stevia leaf are sativoside (5–10% of dry leaf weight), which is about 300 times sweeter than sucrose [5] and rebaudioside A (2–4%), which is more suited than sativoside for use in foods and beverages due to its pleasant taste [44]. It is valuable for diabetics and others to avoid sugar for health reasons. It has many health benefiting plant-derived phyto-chemical compounds that help in controlling blood sugar, cholesterol and blood pressure. In addition to its use as natural sweetener Stevia extracts are further refined for use as table sugar. The product has been added to tea and coffee, cooked or baked goods, processed foods, beverages, it can be safely used in herbal medicines, tonics, for diabetics and in the daily usage products like mouth washes and tooth pastes. It can be used in chocolates and candies not only to meet the market demand by the diabetics, but also to harvest the added advantages of this herb that it does not encourage tooth decay due to its anti-microbial property, unlike the sugar (Sumida 1980). What makes the Stevia plant so special is that it can be used to replace sugar (sucrose), and principal advantages include:

- It is a completely natural product;
- The sweetener, stevioside, contains absolutely no calories;
- The leaves can be used in their natural state;
- Due to its enormous sweetening power, only small quantities need to be used;
- The plant is non-toxic;
- The leaves as well as the pure stevioside extract can be cooked;
- No aftertaste or bitterness;
- Stable when heated up to 200 degrees;
- Non-fermentative;

•Flavour enhancing;

• Herbally it can be used by humans without negative effect

• Non-addictive sweetener for children.

It can then be added in jam, yoghurt, ice creams, smoothies, deserts, chewing gum and sorbets and also to sweeten bitter medicines. Use of stevia as alternative to sugar began in Japan in 1970’s for making food products, soft drinks (Coca Cola), table use, etc. Japan currently consumes more stevia (40% of the sweetener market). Stevia became popular in U.S (Mid 1980’s). Stevia has been successfully adapted to a wide range of climatic locations around the world. It requires a range of rain between 1500 and 1800 mm and temperature extremes of -6^{o}c to +46^{o}c. It is a semi-humid subtropical plant that shows higher leaf production under high light intensity and warm temperature. Day length is more critical than light intensity. Stevia requires very good drainage, any soils that retain the moisture for very long period of time are unsuitable for Stevia cultivation that should be religiously avoided. Similarly, black cotton soils with very heavy clay content should also be avoided. The plant prefers a lightly textured and well-drained soil to which organic matter has been added. Red soil and sandy loam with a pH of 6 to 7 are best for the cultivation of Stevia. Saline soils having a pH value of more than 8 are unsuitable that should not be selected to cultivate this plant.

Rwanda is able to achieve naturally high yields because of sustainable rainfalls, soil, climate conditions and ideal elevation (1400-2000m). It can be a potential source of foreign currency to the country because of high yield which can reach 2t/ha/ year in Rwanda (Stevia life co.Ltd, 2015). It is labor intensive which can create employment opportunities to many Rwandans. Once planted, the stevia plant can be harvested continuously for six years with farmers harvesting four times per year on irrigated land. European regulatory bodies including the joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) have now agreed that steviol glucoside is safe for all populations to consume and is a suitable sweetening option for diabetics. Effective from December 2nd, 2011, the EU has approved its use as a food additive [1]. Due to the short time of stevia introduction as a new crop in Rwanda, there is no information available on nutrient requirement. It is expected that a higher and balanced nutrient supply will result in higher foliage yield. Unfortunately, no detailed study has yet been conducted on fertilizers requirement for large scale cultivation of stevia in Rwanda. Incorporation of stevia into agricultural production systems depends upon details information regarding the plant, its agronomic potential and nutritional requirement (Ramesh et al., 2007). The aim of the present work is to determine the best type of fertilizer to be used for better yield for *stevia rebaudiana* in different agroecological zones of Rwanda.

1.2 Overall objective

The objective of the study is to increase farmers’ income through development of *stevia rebaudiana* production technologies and contribute to human nutrition.

1.2.1 Specific objectives

The objectives of the study include;

- ❖ To develop guidelines for stevia production in Rwanda

- To increase production through promotion and utilization of organic manure in stevia production.

2.1 Materials and Methods

Experiments were conducted during season 2021A in Rubona research station of Rwanda Agriculture and Animal Resources Development Board (RAB) located in southern region of Rwanda and during season 2021B in Ntendrezi research station of Rwanda agriculture and Animal resources Development Board (RAB) located in southern-western region of the country. Ten tons per hectare (10T/ha) of poultry manure, compost, caw dung, pig manure, 200kg/ha of NPK, DAP and 100kg/ha of urea and check (without fertilizers) have been used as treatments. Seedlings of a popular variety of stevia have been raised in nursery bed and transplanted in experimental field at 45 days of age.

Poultry manure (PM) is a valuable organic fertilizer and can serve as a suitable alternate to chemical fertilizer. In agriculture, the main reasons for applying PM include the organic amendment of soil and the provision of nutrients to crops (Warren et al., 2006). PM application registered over 53% increases of N level in the soil, from 0.09 to 0.14 % and exchangeable cations increase with manure application (Boateng et al., 2006).

Cow manure is rich in nutrients and is suitable for plant growth. It has 3% nitrogen, 2% phosphorus, and 1% potassium. 3-2-1 NPK, making it the right type of fertilizer for almost all types of plants and crops. That's because it brings back nutrient balance to fields organically. Being a mixture of feces and urine in the ratio of 3:1, it mainly consists of lignin, cellulose and hemicelluloses. It also contains 24 different minerals like nitrogen, potassium, along with trace amount of sulphur, iron, magnesium, copper, cobalt and manganese.

Pig manure contains all 13 essential nutrients required by plants, including nitrogen (N), phosphorus (P) and potassium (K), as well as a large proportion of organic matter, which provides multiple benefits including: Improves soil texture and quality. Improves water uptake and water holding capacity.

Compost is organic material that can be added to soil to help plants grow. Food scraps and yard waste together currently make up more than 30 percent of what we throw away, and could be composted instead.

Following activities have been realized during experimentation:

2.2 Nursery establishment and management

Before making fertilization experiment, nursery has been established for seedlings production and following techniques were main in nursery establishment and management:

- Raised beds (~15cm height) of 1m in width and 10 m in length are well prepared and edges protected using wood poles. About 30kg of organic manure is mixed in the beds. The bed is drenched with mancozeb (Dithane M45) to prevent rotting of cuttings by fungi and is covered with a transparent plastic sheet for 3 days.
- Before planting, bed watering with about 7 watering cans (arrosoirs) is done.

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- Softwood cuttings, 5cm in height and with at least 2 nodes are taken from the top of young plants still in nursery or older plants in the main field. These cuttings are planted into the beds at the spacing of 5cm between the lines and 2cm in the lines after which watering is done with about 8 watering cans.
- Thereafter, the bed is covered with a transparent plastic sheet at a height of about 50 cm and the sheet net is placed at a nursery structure of about 1.8cm height.

Picture1: Nursery beds covered with plastic sheet

Picture 2: Nursery structure covered by shed net

- 10 days after putting cutting into nursery beds, the transparent plastic sheet is removed step by step during 4days (1/4 of covered area per day) for a progressive aclimatation and seedlings continue to grow up covered by shed net and regular watering is started;
- 20 days after putting cuttings into nursery beds, the shade net is removed and at this time, soil loosening is done in between lines using a pointed stick;
- 30 days after putting cuttings into nursery beds, new cuttings can be taken on their tops to establish another nursery;
- The planting materials are ready to be planted in main field 45 days after putting cuttings into nursery bed

Photo 3: Seedlings at transplanting stage

2.3 Planting and intercultural operations

- The soil is well prepared, weeds and stones are removed.
- Beds of 1.5m width, with convenient length and raised at 10 cm height, are prepared and the distance between 2 beds is 40 cm.

- Stevia plants are uprooted from the nursery, after deep watering, and are planted in the main field at the spacing of 30cm between lines and 20 cm between plants.
- Each nursery bed of $10m^2$ can give 10,000 seedlings and therefore 15 nursery beds, ie.150,000 plants are required to plant 1ha of main field.
- Immediately after planting, watering is done when it is not expected to rain;
- Weeding is done regularly to avoid competition with the main crop;
- In wet period, Dithane M45 is applied to control fungal diseases when noticed;

Stevia, being a vegetatively productive plant mainly needs nitrogen supplementation (Hasnain et al., 2020). Nitrogen is considered as an essential nutrient for foliage growth of plants (Hardjowigeno, 2007).

Using seedlings of popular and cultivated stevia variety, fertilization trial was conducted in Rubona and Ntendezi RAB research stations. Row to row and plant to plant distance was maintained at 20cm and crop was irrigated on need basis. The experiment consisted of 8 fertilization treatments in four replicates totaling 32 experimental plots arranged according to a Randomized Complete Block Design as follows (table 1). Intercultural operations like irrigation, soil loosening, weeding, insect pest control, removal of flowers etc. were done when necessary. Data were collected once a week for plant tillers, plant height and at harvesting for yield. The crop was harvested at 90 days after planting (DAP).

Table 1: Treatments in RCBD

Rep. 1	Rep. 2	Rep. 3	Rep. 4
T 2	T 7	T 8	T 4
T 3	T 3	T 2	T 6
T 5	T 1	T 6	T 7
T 8	T 4	T 7	T 8
T 7	T 6	T 1	T 3
T 6	T 8	T 5	T 1
T 1	T 5	T 4	T 2
T 4	T 2	T 3	T 5

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Where: (T1: compost; T2: cowdung; T3: Pig manure; T4: poultry manure; F5: recommended NPK; F6: recommended DAP; F7: recommended Urea and T8: control treatment (without fertilizer).

Photo 4: Uprooting stevia seedlings **Photo 5: Stevia planting in main field**

2.4 Disease and pest management

In our stevia rebaudiana cultivation trial, diseases affected stevia starting nursery bed until harvest. Among diseases, leaf blight caused by Alternaria sp. and leaf spot caused by Septoria steviae were the most common and mainly occur during rainy season. These diseases reduce stevia leaf yield and quality.

a) Septoria leaf sport of stevia

In the beginning, small brown spot occurred on the leaves and gradually developed to round to rectangle or irregularity expanded, large, brown to dark-brown spots. Symptoms of Septoria steviae begin as small necrotic lesions at the base of the plant that gradually move upward in the canopy under favorable environmental conditions. As the season progresses, lesions coalesce leading to necrosis of entire leaves and eventual defoliation of the canopy. Within the lesions, S. steviae forms structures known as pycnidia that house asexual spores called conidia. As lesions are formed, pycnidia release conidia that can be spread through rain splash throughout the season allowing the pathogen to move up the canopy. Disease spreads most rapidly in periods of high rain fall with cool night temperatures. For this disease management, sanitation in nursery is very essential to reduce inoculum entering new fields because it limits the spread of Septoria leaf spot at this time. The experiment shown that harvesting before environmental conditions become highly conducive for disease progression (harvesting during dry season) can result in higher yields. Eliminate initial source of infection by removing infected plant debris and weeds, and use disease-free seeds. If complete removal of plant debris is not possible, destroy by deep plowing immediately after harvest and follow with a one-year rotation. Preventative fungicide applications or applications in the early stages of disease development provide the best results. Products containing chlorothalonil, mancozeb have been used in our trial and are efficient for good control of stevia Septoria leaf spot disease.

b) Stevia early blight

Stevia early blight has been observed in nursery bed because stevia cuttings are put in very wet conditions in order to stimulate roots initiation. During these conditions, symptoms of blight were rapidly spreading, watery rot of leaves, collapse, shrivel and turn brown. Brown lesions developed also on the leaf stalks (petioles) and stems, white growth was also visible under wet or very humid conditions.

Early blight was managed using Mancozeb 80 % WP, a pesticide commonly called Dithane at a rate of four soup spoons(40g) per 15l of water pump during wet season or three soup spoons per 15l of water pump during dry season. Pesticide application frequency depended on climate conditions and the severity of the disease.

c) Pests

During our experiment it has been observed that young leaves and buds were attacked by caterpillars and chemicals like cypermethrin, deltamethrin were sprayed at a rate of 1.5-2.0 ml /L of water at 15 days interval to manage them.

Pic 6: stevia early blight

Pic 7: Septoria leaf sport of stevia

2.5 Data collection

Observed parameters were plant height, number of tillers, internodes and yield at harvest. Number of tillers and plant height were recorded once per week, internodes length and leaf yield at harvest. Collected data were analyzed using GenStat statistical software

3.1 Result and Discussion

The statistical analysis (ANOVA) for the above parameters was carried out by using Genstat software (table below).

3.2 Tested Fertilizers at Rubona

Table 2: Analysis of variance (ANOVA) of tested fertilizers at Rubona

Fertilizer	Height	Number of Tiller	Yield (T/Ha)	Internode	Thickness	Alternaria spot	Septoria spot
Compost	40.52 a	19.55 ab	3.389 a	0.99ab	1.367	2.25a	5a
Cow dung	40.44 a	17.25 b	4.028 a	1.095a	1.392	1.75a	4a
Pig manure	41.23 a	16.02 b	3.056 a	1.075ab	1.445	2.75a	4.75a
Poultry manure	41.58 a	21.72 a	3.333 a	1.04ab	1.323	2.25a	4.5a
NPK	42.2 a	19.8 ab	3.667 a	1.04ab	1.357	2.25a	4a
DAP	40.66 a	18.9 ab	3a	0.987ab	1.305	2.75a	5.25a
Urea	42.46 a	21.73 a	4.111a	1.007ab	1.347	2.5a	4a
No fertilizer	39.76 a	18.2 ab	3.472a	0.917b	1.323	2.5a	4.5a
CV (%)	12	32.7	29.8	24.8	31	37	34.9
Pvalue	0.199	<.001	0.739	0.059	0.863	0.784	0.906
SE	0.781	0.989	0.522	0.04	0.0666	0.44	0.785
LSD	2.174	2.752	1.534	0.1113	0.1853	1.294	2.308

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3.2.1 Plant Height

During the period of growth, no fertilizer has shown significant effect on plant height. Even though there is no significant effect on plant height maximum plant height has attained with the use of urea. Poultry manure was the second in increasing plant height while the minimum plant height was observed with control treatment.

3.2.2 Number of tillers

The presentation of the number of tillers is summarized in Figure 1

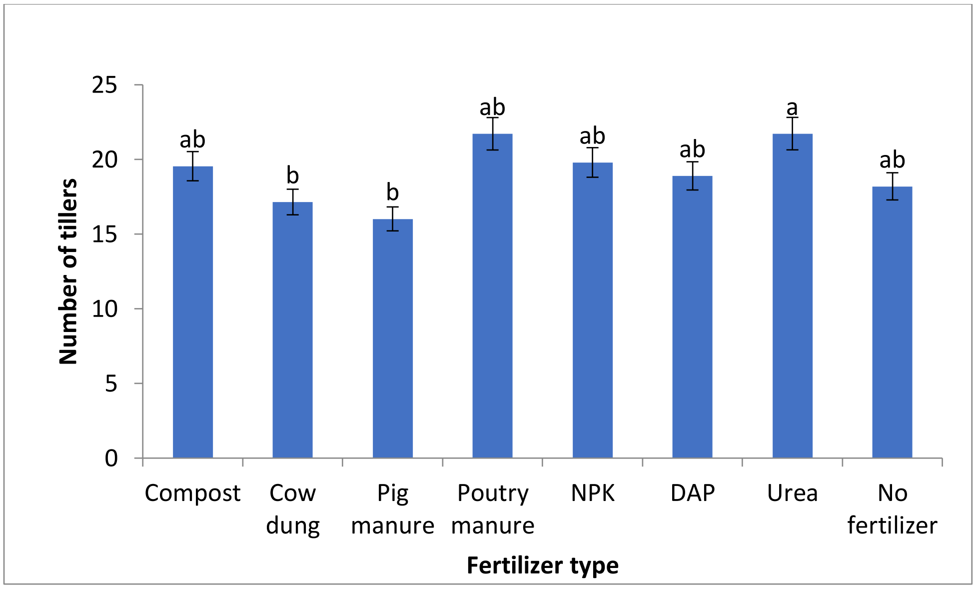

Figure 1: Number of tillers

Significant interaction occurred between the applications of fertilizers and number of tillers. During the observation, urea played more dominant role in increasing number of tillers (21.73) poultry manure was the second (21.72) while control treatment (no fertilizer) showed minimum contribution in increasing number of stevia tillers (18.2)

3.2.3 Internode

Significant interaction occurred between the application of fertilizers and growth between two nodes (internode)

3.2.4 Yield

Plant height and stem diameter of stevia plants were recorded at harvest. The plant height was measured with a meter ruler from ground to the base of the fully opened leaf and the stem diameter was measured with slide calipers up to 0.01 mm accuracy. Biomass yield (total fresh leaf and stem yield), fresh leaf yield, and dry leaf yield were determined in each plant. We estimated the fresh biomass, fresh and dry leaf yield per plant using one digital scale with precision of 0.01 g. Leaves were dried at 50°C temperature in hot air dryer for 6 hours and stored in clean gunny bags. At this temperature, the quality of dried leaves produced, in terms of colour, sweetness and nutrient content, was better compared with drying at 70°C [40]. Dry leaf had an important role in stevia extract in term of quality Fresh Yield of leaves per hectare during the period of harvest has shown no significant interaction between the applications of fertilizers (Table 2). The yield of the harvested fresh leaves of stevia that resulted from urea(100kg/ha) treatment during the period of harvest has shown at least high yield in comparison with other treatments.

3.3 Tested Fertilizers at Ntendezi

Table 3: Analysis of variance (ANOVA) of tested fertilizers at Ntendezi

Fertilizer	Height	Number of Tiller	Yield (T/Ha)	Internode	Thickness	Alternaria spot	Septoria spot
Compost	41.0 a	18.67 ab	3.413 a	0.87ab	1.572	2.25a	4a
Cow dung	43.50b	19.30 b	4.249 b	1.075a	1.492	1.75a	3.9a
Pig manure	42.67 a	20.04 b	3.354 a	1.055ab	1.475	2.75a	4.33a
Poultry manure	44.51 ab	22.76 a	4.744 ab	1.09ab	1.643	2.25a	4.21a
NPK	41.8 ab	18.8 ab	3.623 a	1.05ab	1.270	2.25a	4a
DAP	40.66 a	18.9 ab	3.120a	0.871ab	1.200	2.75a	4.25a
Urea	41.46 a	21.0 a	4.439ab	1.126ab	1.236	2.5a	5.1a
No fertilizer	39.76 a	16.8 ab	3.524a	0.943b	1.022	2.5a	4.5a
CV (%)	11	33.5	26.7	21.5	34	34	35.2
Pvalue	0.197	<.001	0.747	0.061	0.953	0.886	0.809
SE	0.783	0.889	0.626	0.05	0.0764	0.41	0.832
LSD	2.342	2.521	1.614	0.1223	0.1557	1.386	2.509

3.3.1 Plant Height:

Significant interaction occurred between treatments and plant height. During the observation, poultry manure played more dominant role in increasing plant height (44.51) cow dung was the second (43.50) while control treatment (no fertilizer) showed minimum contribution in increasing number of stevia height (39.76)

3.3.2 Number of tillers

Significant interaction occurred between the applications of fertilizers and number of tillers. During the observation, poultry manure played more dominant role in increasing number of tillers (22.76) urea was the second(21.0) while control treatment (no fertilizer) showed minimum contribution in increasing number of stevia tillers(16.8)

3.3.3 Internode

Significant interaction occurred between the application of fertilizers and growth between two nodes(internode)

3.3.4 Yield

Fresh Yield of leaves per hectare during the period of harvest has shown no significant interaction between the applications of fertilizers (Table 3). The yield of the harvested fresh leaves of stevia that resulted from poultry manure has shown at least high yield in comparison with other treatments.

4.1 Conclusion

The results showed there is a significant interaction between the applications of fertilizers in some parameters of growth, such as number of tillers, plant height and internode. It has been showed that for these parameters, plants reacted with treatments. Besides, it was found there is a significant interaction between the application of fertilizers and yield at Ntendezi station while there was no significant interaction between application of fertilizers and yield at Rubona. The application of 100kg/ha of urea has resulted in maximum fresh leaf yield of about 4.111t/ha because urea dissolves very quickly in contact with soil at Rubona

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