Journal of Crop and Weed, 10(1):107-110(2014)

Efficacy of organic manures on growth, yield and biomolecules of stevia (*Stevia rebaudiana* Bertoni)

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Received:18-12-2013, Revised: 5-4-2014, Accepted: 15-4-2014

ABSTRACT

Stevia (Stevia rebaudiana Bertoni) a perennial herb belonging to family Asteraceae is important for its leaves which are used as non caloric sweetner and also in many therapeutic applications including diabetes. The present investigation was carried out during 2012-13 to study the effect of organic nutrition on growth and yield of stevia cv. Meethi. Vermicompost (V.C) -250g, bone meal (B.M.)- 120g and neem cake (N.C.)- 100g were applied as sole and in combination. The experiment comprised ten treatments [T_0:Control; T_1: V.C. (100%); T_2:N.C. (100%); T_3:B.M. (100%); T_4:V.C.+N.C. 75:25); T_5:V.C.+N.C.(50:50); T_6:N.C.+B.M.(75:25); T_7:V.C.+B.M.(50:50); T_8:V.C.+B.M (75:25); T_9:N.C.+B.M.(50:50)] each replicated thrice and followed the randomized block design. The study revealed that organic manures were much more effective for Stevia when they were applied in combination rather than sole application. Under the study, combined application of vermicompost and bone meal at 75:25 ratio showed the maximum height of plant (49.99cm), highest number of suckers per plant (3.52), maximum number of leaves (89.87 plant^{-1}) as well as maximum fresh (26.55g) and dry (7.52g) weight of leaves among the all treatments.

Key words: Biofertilizers, organic manures, stevia, vermicompost.

In general, human health is greatly endangered as various complex diseases developed in the last decade due to excessive intake of "harmful" sugars present in food, beverages and in wide range of food products. From a medical point of view, increased use of products enriched with sugar (sucrose) favour the development of various chronic diseases. Obesity and diabetes are ones of the major diseases of modern mankind and from the health aspect a great emphasis in the prevention thereof was placed. Increased interest of the consumer to reduce sugar intake through the food leads to higher popularization of products that instead of sucrose contain artificial sweeteners. Most artificial sweeteners with a high degree of sweetness are produced from synthetic ingredients or derived exclusively by chemical synthesis in the laboratory. Modern medical researches show that most artificial sweeteners are harmful for human health. Therefore, more and more sweeteners extracted from natural materials are used. Stevioside is natural sweetener isolated from the leaves of plant *Stevia rebaudiana* Bertoni and it is up to 300 times sweeter than sucrose (Noshiyama *et al*., 1991). Dry leaves of stevia are sweeter approximately 10 to 15 times than sucrose while glycemic index is zero, so it is sweetener with no caloric value and with proven non-toxic effect on human health. In recent years, stevia products find widespread use in the food industry (Savita *et al*., 2004). Steviol glycosides are used as a sweetener in many industrial foods, such as soft drinks or fruit juices, desserts, sauces, delicious, sweet corn, bread, biscuits and a table sweetener. Individual tissues of Stevia appear to differ significantly in the stevioside content declining in order: leaves > shoots > roots > flowers. The fact that the highest stevioside content is found in the leaves suggests that they serve as the main tissue for both synthesis and primary accumulation of stevioside compounds. In addition to its sweetening property, it has good medicinal values and uses. Synthetic sugar forms an indispensable ingredient in the food habits of human beings, found to contribute to calories and are not advised for consumption by diabetic patients but stevia is a natural sweetener with low calorie that is used as an alternative to sugar. There is low risk in stevia cultivation as it is an agricultural production; its cultivation is tax free in many regions. Stevia is planted for five years and the returns also come early and are high compared to the other traditional crops. Stevia can be an ideal addition to the organic farming. Randi and Felippe (1981) reported that stevia is used to produce non-caloric sweetener which are natural alternatives to the synthetic sweetening agents. They do not metabolize in the human body. Thus, with the current demand for food supplements having low carbohydrate, minimum calorie and low sugar content, the Stevia plant and its extracts have proven to be the ultimate choice to fight against obesity, cavities, hypertension, fatigue, depression, and also against yeast infection. It possesses hypoglycemic, hypotensive, vasodilating, taste improving, sweetening, anti-fungal, anti-viral, anti inflammatory, anti-bacterial properties and increases urination function of the body (Dyrskog *et al.*, 2005). It has been found to be non toxic, non addictive, non carcinogenic, non mutagenic, non

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teratogenic and is devoid of genotoxic effect. Diabetics (Shock, 1982; Dyrskog *et al*., 2005). It does not affect blood sugar level, hence safe. It is of large industrial and therapeutically value. Stevia still a plant of very recent domestication. It is believed that yield improvements of stevia can be achieved by selection and breeding for stevioside content, leaf to stem ratios and plant response to fertilization. Various aspects relating to organic farming were addressed for sustainable development in horticulture for soil improvement, yield and quality improvement. Manjunatha *et al.* (2013) concluded that organic agriculture can boost soil health and reduce environmental pollution. Hence, there is urgent need of research to establish optimal organic manure dose to achieve higher vegetative growth, high yield and quality improvement in sustainable manner. In this line present investigation was carried out to study the effect of organic nutrients on growth and yield of stevia.

MATERIALS AND METHODS

The experiment was conducted at the Horticulture Research Station, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow (U.P) during the period 2012-13. Geographically this area falls under humid sub-tropical climate and located between 18.60 and 20.20 ^{0}North latitude and 76.00 and 78.00 ^{0}East longitude on an elevation of about 111 meters from sea level in the genetic alluvial plains of eastern Uttar Pradesh, which is subjected to the extreme of weather conditions.. The experimental site has sandy loam alkaline soil having high pH (8.6). The experiment comprised 10 treatments with application of organic manures i.e. Vermicompost (250 gm), Bone Meal (100gm) and Neem Cake (120g) in sole and in combination. There were ten treatment combinations i.e. T_0:Control; T_1: V.C. (100%); T_2:N.C. (100%); T_3:B.M. (100%); T_4:V.C.+N.C. 75:25); T_5:V.C.+N.C.(50:50); T_6:N.C.+B.M.(75:25); T_7:V.C.+B.M.(50:50); T_8:V.C.+B.M (75:25); T_9:N.C.+B.M.(50:50) replicated thrice and laid out under randomized block design. The stevia runners were procured from Central Institute of Medicinal and Aromatic Plants, Lucknow, U.P. The suckers were transplanted on prepared plots at 30 × 30 cm spacing following light irrigation. Weeding was done manually on regular basis. No pesticide or insecticide was applied and no incidence of pest and disease was observed in the experimental field. The leaves were ready for harvesting after five months of planting. Various growth (plant height, circumference, number of suckers, number of primary branches) and yield parameters (number of leaves, fresh and dry weight of yield) were observed and data were recorded from randomly selected 5 plants form each block. The observed data were analyzed using analysis of variance as stated by Panse and Sukhatme (1985). Fourier Transform Infra-Red (FTIR) spectroscopy was done to see the changes in effective biomolecules. The leaves were shade dried in room temperature for 14 days and powdered in a grinder to a fine powder form. Powdered was mixed with completely dried potassium bromide (at a ratio of 1/100), and the mixture of was subjected to a pressure of 5×10^6 pa in an evacuated die to produce a Kbr pellet for use in a FTIR spectrometer. FTIR spectra were recorded with a FTIR 460 plus Jasco. The powdered samples were mixed with dried potassium bromide and prepared as pallets, scanned at room temperature (25±2^{o}C) at 4000-400 per cm. spectral range to imperative synthesize to ratio for each spectrum, 100 interferograms with a special resolution of ±4 per cm were average. Background spectra, which were collected under identical conditions, were substracted from the sample spectra. Each sample was scanned under the same condition with 10 different pallets. Special care was taken to prepare the pallets at the same thickner by taking the same amount of sample and applying the same pressure. Therefore, in the present it was directly relate the intimates of the absorption bands to the concentration of the corresponding functional groups.

RESULTS AND DISCUSSION

The effect of organic manures in alone as well as combination treatments improved the growth and yield of *Stevia rebaudiana* cv. Meethi. Among all the treatments, the best results was shown by the combinations of Vermicompost + Bone Meal. Table1 showed that the treatment T_8 (Vermicompost + Bone meal at 75: 25 ratio) had better effect on plant height at different days after transplanting. The plants under treatment T_8 had the maximum height (49.99 cm) at maturity i.e. at 150 days after transplanting (DAT) followed by T_1 (100 % Vermicompost). This trend was also noticed at 30, 30, 60, 90, 120 DAT where application of vermicompost + bone meal at 75:25 ratio showed the highest plant height. This result was also in line of work of Umesha *et al*. (2011).

Table 1 also showed that organic supplements had significant effect on circumference on stevia. According to the experimental results, maximum (2.47 cm) circumference was recorded under treatment T_8. The experimental findings showed that the treatment T_8 increased the basal circumference at 30 DAT and found similar trend in improvement of circumference at 60, 90, 120 and 150 DAT. At all the stages, the increase was statistically significant. The treatment T_1 also showed increase in circumference followed by T_8 and the lowest was recorded under T_0

treatment (control). The increase in circumference was might be due to better combination of vermicompost and bone meal. The result was also supported by Dube (2011).

There was significant effect on number of suckers at transplanting on stevia due to organic manures and the effect due to T_8 was found better in respect of sucker production which produces maximum suckers per plant i.e. beneficial for production of planting material for the next season. The finding was also corroborated with works of Ramasamy and Suresh (2010). It was also seen that the plants under treatment T_8 had the maximum number of primary branches (Table- 2). This might be due to application of good organic source which enables very good air movement of soil moisture and environmental factors. Khatik and Dikshit (2001) also found the similar trend. It was observed that the number of leaves per plant increased

Table 1 : Effect of organic nutrient on plant height, circumference and sucker production of stevia

Treatment	Plant height (cm)					Circumference (cm)					Number of suckers plant^{-1}				
	DAT					DAT									
	30	60	90	120	150	30	60	90	120	150	30	60	90	120	150
T_0 Control	8.98	18.99	36.00	39.00	45.38	0.99	1.27	1.51	1.73	1.82	1.03	1.22	1.41	1.72	1.94
T_1 V.C. (100%)	11.39	24.19	42.80	45.73	49.13	1.58	1.83	2.01	2.18	2.22	1.57	2.49	2.92	3.30	3.34
T_2 N.C. (100%)	9.06	20.87	42.67	42.86	48.07	1.24	1.37	1.57	1.87	1.90	1.27	2.31	2.62	2.74	2.78
T_3 B.M. (100%)	10.88	21.39	37.60	44.66	48.20	1.24	1.44	1.52	2.00	2.12	1.04	2.45	2.46	2.63	2.71
T_4 V.C.+N.C.(75:25)	11.20	22.25	36.20	43.67	45.46	1.52	1.68	1.74	1.85	2.03	1.07	1.53	1.71	2.08	2.22
T_5 V.C.+N.C.(50:50)	10.05	19.95	38.67	41.07	48.52	1.29	1.78	1.85	1.96	2.07	1.08	1.82	2.16	2.38	2.58
T_6 N.C.+B.M.(75:25)	9.72	22.07	37.17	43.60	46.9	1.20	1.80	2.00	2.06	2.09	1.53	2.58	2.68	3.04	3.12
T_7 V.C.+B.M.(50:50)	9.35	20.27	39.80	43.39	46.87	1.04	1.57	1.63	1.78	2.08	1.15	1.52	1.74	1.93	2.00
T_8 V.C.+B.M(75:25)	11.67	26.13	44.20	46.13	49.99	1.85	2.07	2.21	2.26	2.47	1.79	2.92	3.38	3.41	3.51
T_9 N.C.+B.M.(50:50)	11.00	23.73	39.27	41.57	46.93	1.55	1.72	1.80	2.00	2.08	1.41	1.51	2.64	2.71	2.76
SEm(±)	**0.419**	**1.953**	**1.855**	**1.232**	**1.400**	**0.209**	**0.318**	**0.299**	**0.315**	**0.299**	**0.266**	**0.360**	**0.469**	**0.520**	**0.532**
LSD (0.05)	**0.880**	**3.200**	**3.000**	**3.690**	**3.040**	**0.440**	**0.670**	**0.630**	**0.660**	**0.630**	**0.560**	**0.760**	**0.990**	**1.090**	**1.120**

Table 2: Effect of organic manuring on yield and yield attributing characters of stevia.

Treatment	Number of primary branches plant^{-1}					Number of leaves plant^{-1} (g)					FW (g)	DW (g)
	DAT					DAT						
	30	60	90	120	150	30	60	90	120	150		
T_0:Control	1.23	1.41	1.78	2.07	2.13	10.73	20.13	34.13	70.93	81.33	21.17	6.51
T_1: V.C. (100%)	1.47	1.99	2.40	3.09	3.27	14.87	23.96	41.47	80.93	87.60	25.19	7.43
T_2:N.C. (100%)	1.40	1.88	1.90	2.41	2.70	12.87	22.53	38.53	79.33	81.73	21.30	6.03
T_3:B.M. (100%)	1.42	1.49	2.19	2.13	2.22	10.73	20.67	41.07	77.33	83.07	24.45	6.45
T_4:V.C.+N.C. 75:25)	1.47	1.71	1.89	2.78	2.81	11.87	20.13	40.27	73.20	82.67	23.86	6.69
T_5:V.C.+N.C.(50:50)	1.32	1.44	2.24	2.51	2.58	15.93	21.20	37.20	79.00	82.80	23.51	6.47
T_6:N.C.+B.M.(75:25)	1.21	1.58	1.94	2.83	3.02	12.07	20.54	38.67	76.93	86.00	24.26	6.53
T_7:V.C.+B.M.(50:50)	1.41	1.79	1.86	2.85	2.90	12.87	23.73	38.53	73.47	84.00	23.35	6.33
T_8:V.C.+B.M (75:25)	1.48	2.25	2.55	3.41	3.58	16.27	24.67	42.93	82.80	89.87	26.55	7.52
T_9:N.C.+B.M.(50:50)	1.27	1.49	1.53	2.70	2.74	11.27	22.32	37.73	78.13	89.43	23.93	6.88
SEm(±)	**0.277**	**0.303**	**0.445**	**0.455**	**0.441**	**1.304**	**2.836**	**1.116**	**1.470**	**1.281**	**0.202**	**0.424**
LSD(0.05)	**0.580**	**0.640**	**0.940**	**0.960**	**0.930**	**2.740**	**5.960**	**2.650**	**2.890**	**3.290**	**0.426**	**0.890**

V.C. -Vermicompost, B.M.- Bone Meal, N.C.-Neem Cake, DAT- Days after transplanting, F.W. - Fresh weight of leavs, D.W. - Dry weight of leaves

continuously from 30 to 150 DAT, but the rate of increase was decreased after 120 DAT and the number of leaves was ranged from 81 to 89 per plant at 150 DAT under various treatments. Among the treatments, T_8 followed by T_9 had the maximum number of leaves per plant. The yield and yield attributes in terms of fresh weight, dry weight of leaves per plant was recorded in Table 2. Higher fresh leaf yield and dry leaf yield of stevia was noted with application of higher Vermicompost, Bone meal, and their combination and T_8 (Vermicompost + Bone meal at 75: 25 ratio) had the maximum leaf yield. Their nutrient levels could be attributed to more number of branches and leaves of per plant of stevia due to higher plant height. It was also experimentally proved that no manuring resulted in lowest leaf yield of stevia. Increase in dry leaf yield with organic manuring was also reported by Shock (1982).

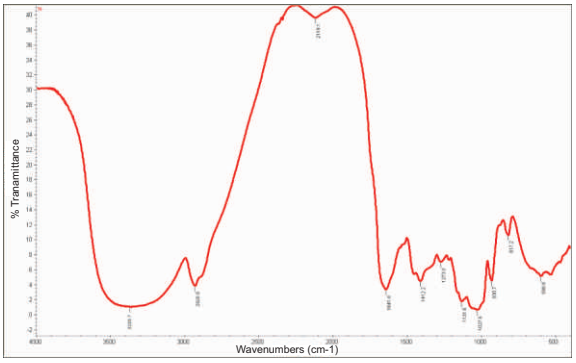

Fig.1: FTIR spectrograph for effect of organic nutrients on growth yield quality of stevia (T_8)

The result of functional groups analysis using FTIR analysis (data not shown) revealed the existence of various biochemical and nutritive values in stevia which proved that application of organic nutrients was better for improvement of bio-molecules present in stevia leaves.

The present study showed that the vegetative growth, multiplication of suckers, leaf yield and quality of stevia have been very much influenced by the application of organic nutrients. From the present study, it may be concluded that the application of vermicompost and bone meal at 75:25 ratio was proved to be the best for improving the growth, yield and quality (in terms of effective bio-molecules) of stevia cv. Meethi under Lucknow condition.

Table 3: Effective biomolecules present in stevia as influenced by the best treatment T_8

Wave length (µm)	Frequency(cm^{-1})	Bond	Type of compound
3289.7	3000-3700	N-H	Amines
2928.9	2850-2960	C-H	Alkenes
2119.1	2100-2660	-C=C-	Alkynes
1641.6	1620-1680	>C=C>	Alkenes
1412.2	1330-1560	$-NO_2$	Nitro
1273.0	1180-1360	-C-N-	Amines
1131.6	1000-1300	-C-O-	Alcohols, Ethers
1027.6	1000-1300	-C-O-	Alcohols, Ethers
930.7	600-1500	-C-O-	Alcohols, Ethers
817.2	600-1500	C-C-	Alkenes
598.8	500-600	C-Br	Alkenes

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