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Organic manures and bio-fertilizers effectively improve yield and quality of stevia (*Stevia rebaudiana*)

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Abstract

The influence of different organic manures, bio-fertilizers and green manures on growth, yield and glycoside content of stevia (*Stevia rebaudiana*) was studied over a period of three years (2004-2007). Various organic supplements *viz.*, farm yard manure (15 and 25 t ha^{-1}), vermicompost (1 and 2 t ha^{-1}), neem cake (0.5 and 1 t ha^{-1}) and bio-fertilizers *viz.*, *Azospirillum*, phosphorus solubilizing bacteria and VAM (each @ 0 and 10 kg ha^{-1}) were applied. The results of eleven harvests revealed that all the growth parameters *viz.*, plant height, number of branches and plant spread were influenced by various organics and bio-fertilizers and showed variation from season to season (harvest to harvest) and plants responses did not followed a definite trend. Dry leaf yield during first (6.16 t ha^{-1}) and second year (4.34 t ha^{-1}) of cropping was maximum with the treatment receiving FYM (25 t ha^{-1}) + vermicompost (2 t ha^{-1}) + neem cake (1 t ha^{-1}) + bio-fertilizers (10 kg ha^{-1}) and differed significantly. However, in third year of cropping the treatments had no significant influence on the dry leaf yield. Both the glycosides *i.e.*, stevioside (7.8 %) and rebaudioside content (3.4 %), and glycoside yield were also highest in the above said treatment.

Key words: Bio-fertilizers, glycoside content, green manures, organic manures, quality, stevia, yield.

Introduction

Stevia (*Stevia rebaudiana* (Bertoni) Hemsl.) is a sweet herb belonging to Asteraceae family and is a natural non caloric bio-sweetener, which offers a solution for complex diabetic problems and obesity in humans. Worldwide demand for high potency bio-sweeteners is expected to increase significantly in the years to come (Ramesh *et al.,* 2006). The leaves of stevia contain around ten sweetening glycosides, of which stevioside (3-10%), rebaudiside-A (13%), rebaudiside-B, rebaudiside-C, and rebaudiside-D are important. The sweetener imparts 250 times more sweetness than table sugar and 300 times more than sucrose. It has become a potential alternative source by replacing artificial sweeteners like saccharin, aspartame, asulfam-K *etc*. It is estimated that by 2030, Indian's contribution to the diabetic global population would be a whopping 79.4 million – the largest in the world (*www.indiavision.com*). With such a huge share of the population being diabetic, the new ventures in the food industry are focused entirely on non caloric, natural sweeteners safe to diabetics (Bharathi, 2003). In this regard, stevia could be used in the preparation of herbal medicines and tonics not only for diabetics but also for normal people owing to its other advantages.

According to Indian system of medicine *viz.*, *Ayurveda, Unani* and *Siddha,* the raw materials obtained from medicinal herbs should be free from any toxic or chemical residues. In modern agriculture, the use of agro chemicals is intensified resulting in their residues in the medicinal preparations. With the indiscriminate use of chemical fertilizers and pesticides, there is also an increasing risk of health hazards and environmental pollution. In case of medicinal plants, it is the need of the hour to switch on to the sustainable, eco-friendly and safe organic farming system. Even though, the nutrient uptake study has not been done for this crop, the recommended dose of fertilizers is estimated to be 60:30:45 kg NPK ha^{-1} under Bengaluru conditions (Farooqi and Sreeramu, 2004). However, not much work has been done for supplementing the inorganic source of nutrients with organics without affecting the productivity and quality of stevia and hence the present study was carried out with organic nutrition.

Materials and methods

The experiment was conducted at the Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru, during August, 2004 to April, 2007. The experimental site is situated at an elevation of 930 m above MSL, 12°58" N latitude and 77°55" E longitude. The site has red sandy loam soil with 6.72 pH, 0.3 dsm^2 EC, 0.73% organic carbon and 298, 35.7 and 176.5 kg of available NPK ha^{-1}. A mean of 28.96 °C maximum temperature, 17.92 °C minimum temperature, 69.27% RH and 7.08 bright sunshine hours was recorded during the period of experimentation. The experiment was laid out in RCBD design with 18 treatments repeated thrice which consisted of different levels and combinations of organic manures *viz.*, farm yard manure (15 and 25 t ha^{-1}), vermicompost (1 and 2 t ha^{-1}), neem cake (0.5 and 1 t ha^{-1}) and three bio-fertilizers (*Azospirillum brazilense,* PSB - *Bacillus megaterium* and VAM - *Glomus bagyaraji* each @ 10 kg ha^{-1}). Treatments consisting of green manuring with horse gram (T_{17}) and cow pea (T_{18}) were also included. The experimental field was thoroughly prepared and divided into gross plots of 3.6 x 2.1 m.

The recommended dose of fertilizers for stevia is 60:30:45 kg

Table 1. Nutrient contents of different organic manures used in the study

Name of the organic manure	N (%)	P (%)	K(%)
Farm Yard Manure	0.47	0.34	0.31
Vermicompost	1.32	0.28	1.46
Neem cake	2.08	0.60	0.63

NPK ha^{-1} under Bengaluru condition. The organic manures were analyzed for their nutrient contents (Table 1) and organic manures required for individual treatment were calculated on P basis. Different organic manures and bio-fertilizers were combined in such a way that each treatment would receive the basic nutrient requirement. About 40% of FYM and vermicompost were applied according to the treatment combinations as basal dose at the time of land preparation. Whereas, 50% of the recommended dose of neem cake and full dose of bio fertilizers were applied at the time of planting by mixing it with half a kilogram of FYM. The remaining doses of organic manures were divided into three equal splits and were applied after every harvest (*i.e.* at three months interval). In second and subsequent years after each leaf harvest, 25% of the recommended dose was applied. Green manure crops *viz.,* horse gram and cow pea were sown one month prior to stevia planting. One and half month old green manure crops were incorporated in the furrows before flowering and mixed well into the soil manually. Soil incorporation of horse gram and cow pea have added 60 and 66 kg N ha^{-1}, respectively. The same procedure was repeated in subsequent years during the onset of monsoon.

Three months old, healthy, vigorous and uniform rooted cuttings of local variety were planted in the field adopting a spacing of 45 × 30 cm during August 2004. Intercultural operations such as weeding, irrigation, earthing-up and de-flowering were carried out at regular intervals as per recommendation for the crop. During the cropping period, infestation of termites was noticed. The affected plants were removed and destroyed and the affected area was drenched with neem based pesticide, Neem gold at 5 mL L^{-1}.

The first harvest of the crop was done after ninety days of planting and subsequent harvests (ratoon crops) were taken at every three months interval. In total eleven harvests were taken during the entire cropping period. Harvesting was done by cutting plants 15 cm above ground level using sharp sickle. At the end of third year as there were too many gaps due to termite problem and *Septoria* leaf spot, twelth harvest could not be obtained. The leaves were separated from the twigs and then shade dried for one week till the crackling and crisping sound was observed and the leaves were powdered by using mixer grinder. The stevioside, rebaudiside and total glycoside content was analysed by HPLC only for pooled samples of I year. The data was analyzed statistically at P=0.05 using standard statistical procedure (Gomez and Gomez, 1984).

Results and discussion

Growth parameters: The plant height differed significantly due to application of different organic manures at varied levels and bio-fertilizers during I (2004-05) and II (2005-06) years while, the differences were non-significant during third year (2006-07) of cropping (Table 2). Taller plants were observed during first year compared to subsequent two years of cropping, as the vigour was found to decrease from first to subsequent years. The perusal of data indicates that in general, higher organic matter resulted in taller plants compared to the lower levels. However, no definite trend could be observed due to the application of bio-fertilizers. Maximum plant height was recorded in T_{11} (FYM 25 t+ VC 1.0 t+ NC 1.0 t) at 6 and 12 months after planting (MAP), while at 9 and 21 MAP, tallest plants were observed in T_{16} (FYM 25 t + VC 2.0 t + NC 1.0 t + BF 10 kg ha^{-1}) (93.7 and 45.6 cm, respectively). Whereas, T_{12} resulted in maximum plant height at 15 MAP (48 cm).

The stevia plants responded well to ratooning (Table 3) as number of branches steadily increased from 3 MAP (I harvest) to 9 MAP (III harvest) and later vigour decreased due to lack of rejuvenation capacity as reported by Charan Kumar (2009). The

Table 2. Influence of different organic manures and bio-fertilizers on plant height (cm) of stevia (*S. rebaudiana* Bert.)

Treatments	2004-05				2005-06				2006-07		
	3 MAP	6 MAP	9 MAP	12 MAP	15 MAP	18 MAP	21 MAP	24 MAP	27 MAP	30 MAP	33 MAP
T_1 – FYM 15 t + VC 1.0 t + NC 0.5 t / ha	41.4	41.4	72.2	59.0	38.3	40.2	39.5	39.9	40.1	44.6	49.7
T_2 – FYM 15 t + VC 1.0 t + NC 0.5 t + BF/ha	39.6	37.7	61.9	57.7	35.1	40.1	37.9	35.3	34.1	45.2	53.4
T_3 – FYM 15 t + VC 1.0 t + NC 1.0 t / ha	43.4	45.3	63.7	58.3	44.1	37.4	39.5	35.4	36.6	43.1	51.7
T_4 – FYM 15 t + VC 1.0 t + NC 1.0 t + BF/ha	42.1	39.0	63.1	57.1	41.8	38.9	40.5	40.9	40.1	43.2	52.3
T_5 – FYM 15 t + VC 2.0 t + NC 0.5 t / ha	43.5	58.9	79.3	55.3	35 .1	39.4	37.9	39.1	33.2	45.1	53.8
T_6 – FYM 15 t + VC 2.0 t + NC 0.5 t + BF/ha	39.4	48.4	75.1	62.1	35.3	38.3	35.4	32.6	36.5	43.1	45.9
T_7 – FYM 15 t + VC 2.0 t + NC 1.0 t / ha	42.3	48.9	68.6	59.3	29.1	33.6	32.6	35.0	33.5	40.0	42.9
T_8 – FYM 15 t + VC 2.0 t + NC 1.0 t + BF/ha	43.1	59.5	58.4	61.1	43.4	37.9	40.7	40.7	31.9	40.2	53.6
T_9 – FYM 25 t + VC 1.0 t + NC 0.5 t / ha	38.8	42.8	69.7	64.3	45.3	40.5	41.2	37.7	43.1	44.9	42.3
T_{10} – FYM 25 t + VC 1.0 t + NC 0.5 t + BF/ha	43.5	41.8	72.2	63.7	41.7	39.7	40.1	38.9	39.3	45.2	45.6
T_{11} – FYM 25 t + VC 1.0 t + NC 1.0 t / ha	45.9	60.1	71.6	73.9	37.6	39.8	39.4	40.9	29.8	44.3	49.5
T_{12} – FYM 25 t + VC 1.0 t + NC 1.0 t + BF/ha	40.3	54.6	78.1	60.0	48.0	37.3	44.5	48.1	41.0	43.2	45.4
T_{13} – FYM 25 t + VC 2.0 t + NC 0.5 t / ha	40.4	46.5	91.7	59.0	38.6	39.8	40.1	42.0	33.2	44.1	51.3
T_{14} – FYM 25 t + VC 2.0 t + NC 0.5 t + BF/ha	42.8	51.3	89.1	61.3	45.3	38.3	41.5	40.9	34.4	48.7	50.1
T_{15} – FYM 25 t + VC 2.0 t + NC 1.0 t / ha	40.9	43.4	92.9	59.8	38.7	38.1	38.8	39.5	38.3	44.2	42.7
T_{16} – FYM 25 t + VC 2.0 t + NC 1.0 t + BF/ha	39.5	48.5	93.7	69.7	46.1	38.3	45.6	52.3	44.9	45.6	46.7
T_{17} – Green manuring with horse gram	45.2	48.6	81.2	46.5	41.3	39.9	41.0	41.8	28.4	47.5	43.7
T_{18} – Green manuring with cow pea	41.0	47.5	61.6	43.8	36.2	39.9	38.2	28.5	29.7	48.8	49.9
S Em±	2.5	4.0	3.1	3.6	2.6	2.3	1.9	4.7	4.2	2.5	3.4
CD (P=0.05)	-	11.5	8.8	10.3	7.5	-	5.5	-	-	-	-

FYM – Farm yard manure; VC – Vermicompost; NC – Neem cake; BF – Bio-fertilizers (*A. brazilense,* PSB-*B. megaterium* and VAM - *G. bagyaraji*) each @ 10 kg ha^{-1}; *MAP – months after planting

treatments T_{10}, T_{16} and T_{13} had maximum number of branches at 9, 12 and 21 MAP (55, 20.2 and 19.9 plant^{-1}, respectively), which was found to be *at par* with T_9, $T_{16,}$ T_{15}, T_8 and T_{10}. The lowest number of branches was observed in T_2, T_{18} and T_3 (33.06, 9.18 and 10 plant^{-1} respectively). Similarly, plant spread recorded significance differences during first year and the influence was not significant during second and third year of cropping except at 21 MAP during second year (Table 4). Maximum plant spread was recorded in T_{13} with a canopy size of 3,639 cm^2 at second harvest, while T_{16} resulted in highest plant spread at 9 and 12 MAP (2064.3 and 3467.6 cm^2). At 21 MAP, plants of T_{12} treatment had maximum plant spread (1749 cm^2). Plots received green manures only, recorded minimum plant spread at 12 and 21 MAP.

In general, the treatments which included combined application of higher doses of FYM, vermicompost and neem cake along with bio-fertilizers were optimum in increasing different growth parameters of stevia. The maximum growth obtained may be attributed to the role of organic manures and bio-fertilizers in better mobilization of plant nutrients that led to vigorous growth of plants in these treatments. Charan Kumar (2009) in stevia, Joy *et al.* (2005) in *Curculigo orchioides* and Sudhakara (2005) in *Coleus barbatus* also recorded that the use of organic manures alone or in combination improved growth parameters of different medicinal plants.

Table 3. Influence of different organic manures and bio-fertilizers on number of branches in stevia (*S. rebaudiana* Bert.)

Treatments	2004-05				2005-06				2006-07		
	3 MAP	6 MAP	9 MAP	12 MAP	15 MAP	18 MAP	21 MAP	24 MAP	27 MAP	30 MAP	33 MAP
T_1 – FYM 15 t + VC 1.0 t + NC 0.5 t / ha	9.5	15.7	38.4	10.0	17.9	15.7	14.1	8.7	6.20	11.53	11.40
T_2 – FYM 15 t + VC 1.0 t + NC 0.5 t + BF/ha	9.0	19.9	33.1	11.0	21.5	17.5	15.7	8.2	6.78	10.87	9.47
T_3 – FYM 15 t + VC 1.0 t + NC 1.0 t / ha	9.5	28.3	40.1	9.9	13.3	10.8	10.0	5.8	5.97	12.67	9.47
T_4 – FYM 15 t + VC 1.0 t + NC 1.0 t + BF/ha	10.9	24.1	38.0	9.9	17.3	15.1	13.7	8.8	7.60	11.53	9.33
T_5 – FYM 15 t + VC 2.0 t + NC 0.5 t / ha	11.1	21.7	41.3	10.8	19.3	17.5	16.5	12.6	7.57	10.93	9.13
T_6 – FYM 15 t + VC 2.0 t + NC 0.5 t + BF/ha	9.6	20.9	38.9	12.8	15.5	17.7	13.6	7.6	6.87	11.47	10.27
T_7 – FYM 15 t + VC 2.0 t + NC 1.0 t / ha	9.8	23.7	34.1	10.5	23.1	18.1	16.6	8.7	8.65	11.93	10.47
T_8 – FYM 15 t + VC 2.0 t + NC 1.0 t + BF/ha	9.4	25.5	37.7	12.9	25.5	18.5	17.3	8.0	7.83	10.07	9.07
T_9 – FYM 25 t + VC 1.0 t + NC 0.5 t / ha	9.9	28.8	37.1	15.5	24.9	20.9	18.9	10.9	7.75	12.33	12.80
T_{10} – FYM 25 t + VC 1.0 t + NC 0.5 t + BF/ha	9.7	20.7	55.0	16.6	24.5	15.7	17.0	10.9	6.27	9.93	8.47
T_{11} – FYM 25 t + VC 1.0 t + NC 1.0 t / ha	11.1	30.9	40.2	13.6	24.6	17.2	16.9	8.8	7.80	12.20	6.93
T_{12} – FYM 25 t + VC 1.0 t + NC 1.0 t + BF/ha	9.8	20.3	45.3	13.8	19.3	16.5	15.5	10.7	8.03	13.27	12.60
T_{13} – FYM 25 t + VC 2.0 t + NC 0.5 t / ha	9.6	29.3	37.9	11.6	33.5	16.5	19.9	9.7	9.27	10.00	11.00
T_{14} – FYM 25 t + VC 2.0 t + NC 0.5 t + BF/ha	9.0	18.5	38.8	11.9	20.5	19.1	17.0	11.5	7.90	13.20	10.73
T_{15} – FYM 25 t + VC 2.0 t + NC 1.0 t / ha	8.5	20.7	42.3	12.4	20.6	21.7	17.8	11.0	9.48	11.07	12.00
T_{16} – FYM 25 t + VC 2.0 t + NC 1.0 t + BF/ha	8.4	26.0	48.7	20.2	21.4	16.6	17.0	12.9	8.90	13.00	7.27
T_{17} – Green manuring with horse gram	8.0	25.3	37.7	11.9	22.7	12.8	15.7	11.6	7.03	12.07	9.13
T_{18} – Green manuring with cow pea	7.4	15.8	39.7	9.2	12.1	14.0	11.3	7.9	7.67	12.27	11.40
S Em±	1.2	31.2	3.1	1.7	4.6	3.4	1.7	1.8	1.10	1.60	1.70
CD (*P*=0.05)	-	-	8.9	4.8	-	-	-	-	-	-	-

FYM – Farm yard manure; VC – Vermicompost; NC – Neem cake; BF – Bio-fertilizers (*A. brazilense,* PSB-*B. megaterium* and VAM - *G. bagyaraji*) each @ 10 kg ha^{-1}; *MAP – months after planting

Table 4. Influence of different organic manures on plant spread area (sq cm) in stevia (*S. rebaudiana* Bert.)

Treatments	2004-05				2005-06				2006-07		
	3 MAP	6 MAP	9 MAP	12 MAP	15 MAP	18 MAP	21 MAP	24 MAP	27 MAP	30 MAP	33 MAP
T_1 – FYM 15 t + VC 1.0 t + NC 0.5 t / ha	1464	1506	1136	2450	2345	1785	1576	597	579	3887	1678
T_2 – FYM 15 t + VC 1.0 t + NC 0.5 t + BF/ha	1442	1904	818	2166	1734	1488	1249	523	538	3561	1634
T_3 – FYM 15 t + VC 1.0 t + NC 1.0 t / ha	1580	2268	721	2012	1938	1552	1316	458	263	3726	1765
T_4 – FYM 15 t + VC 1.0 t + NC 1.0 t + BF/ha	1688	2364	1060	2748	1745	1472	1240	504	479	3697	1387
T_5 – FYM 15 t + VC 2.0 t + NC 0.5 t / ha	1563	2439	1081	2495	1957	2127	1597	708	567	4183	1701
T_6 – FYM 15 t + VC 2.0 t + NC 0.5 t + BF/ha	1607	2072	1032	2634	1730	1522	1195	332	718	4573	1574
T_7 – FYM 15 t + VC 2.0 t + NC 1.0 t / ha	1593	2165	589	2132	1712	1479	1243	538	566	3927	1136
T_8 – FYM 15 t + VC 2.0 t + NC 1.0 t + BF/ha	1571	2235	908	2655	2235	1731	1451	378	453	2752	1340
T_9 – FYM 25 t + VC 1.0 t + NC 0.5 t / ha	1594	2451	1159	2561	2364	1898	1604	549	324	4045	1926
T_{10} – FYM 25 t + VC 1.0 t + NC 0.5 t + BF/ha	1553	2620	953	2463	2396	1675	1513	468	363	3478	1283
T_{11} – FYM 25 t + VC 1.0 t + NC 1.0 t / ha	1677	3527	1481	2581	1969	1596	1390	606	401	3636	1734
T_{12} – FYM 25 t + VC 1.0 t + NC 1.0 t + BF/ha	1403	2744	979	2781	2727	1902	1749	618	363	4608	1659
T_{13} – FYM 25 t + VC 2.0 t + NC 0.5 t / ha	1527	3639	740	2523	2114	1798	1505	604	571	4316	2164
T_{14} – FYM 25 t + VC 2.0 t + NC 0.5 t + BF/ha	1435	2212	1439	2432	2373	1758	1555	533	596	4809	1553
T_{15} – FYM 25 t + VC 2.0 t + NC 1.0 t / ha	1379	1442	854	2627	2129	1534	1473	776	444	5032	1396
T_{16} – FYM 25 t + VC 2.0 t + NC 1.0 t + BF/ha	1627	2435	2064	3468	2161	1468	1455	737	508	4888	1337
T_{17} – Green manuring with horse gram	1569	2920	780	1834	2369	1493	1496	625	664	5324	1087
T_{18} – Green manuring with cow pea	1213	2656	690	1660	1578	1121	1020	362	400	4821	1637
S Em±	183	368	137	231	287	167	105	154	118	687	249
CD (*P*=0.05)	-	1058	393	664	-	-	301	-	-	-	-

FYM – Farm yard manure; VC – Vermicompost; NC – Neem cake; BF – Bio-fertilizers (*A. brazilense,* PSB-*B. megaterium* and VAM - *G. bagyaraji*) each @ 10 kg ha^{-1}; *MAP – months after planting

FYM is known to increase crop yield by its favourable effect on physical, chemical and biological factors that determine productivity and fertility status of soil and supply nutrients in their available form. Whereas, vermicompost besides, increasing the population of microbes also provides sufficient energy for them to remain active. It also provides the vital macro-nutrients such as N, P, K, Ca, Mg and micronutrients such as Fe, Zn, Cu, Mn and Mo. Besides, neem cake which has nitrification property helps the plants for better uptake of nutrients and consequently the good growth of plants. Incorporation of organic manures not only reduces bulk density but also improves the soil porosity, hydraulic conductivity and water holding capacity besides adding required nutrients (Maheswarappa *et al.,* 1999). *Azospirillum* is well known for its capacity to increase plant growth by fixing the atmospheric nitrogen and also to produce growth promoting substances like hetero-auxins and gibberellins, while PSB increases plant growth by increasing phosphorus solubilization. VAM fungus increases uptake of phosphorus and other minor elements like Zn and Cu. Further, VAM fungi are known to increase water uptake also (Manjunatha *et al.,* 2002).

Yield parameters: Data indicated significant differences among the treatments with respect to dry leaf yield during first and second years of cropping (Table 5), wherein T_{16} recorded the highest dry leaf yield (6163.9 and 3535.8 kg ha^{-1}, respectively). Whereas, the lowest dry leaf yield was recorded in T_{14} and T_7 during first and second years (2960.80 and 2159.6 kg ha^{-1}, respectively). There was a significant difference in dry leaf yield per hectare at 3, 12 and 18 MAP. At 3 MAP, T_4 recorded the highest dry leaf yield (592.59 kg ha^{-1}) which was *at par* with most of the treatments combinations and differed significantly from green manure (T_{17} and T_{18}). The lowest dry leaf yield was registered in T_{17} (303.39 kg ha^{-1}). At fourth harvest, maximum dry leaf yield was recorded in treatment T_{16} (2030.86 kg ha^{-1}), which was *on par* with T_{15} and lowest was observed in T_{18} (753.08 kg ha^{-1}). At sixth harvest, T_8 registered the maximum dry leaf yield (919.5 kg ha^{-1}), which was *on par* with T_{13}, T_{12} and $T_{2,}$ while the lowest was in T_7 (436.94 kg ha^{-1}). The results of the present study are in line with the findings of Charan Kumar (2009) and Eranna (2007) in stevia.

The herbage yield is completely dependent on the growth attributes throughout the life cycle of the crop. The higher dry leaf yield with higher levels of organic manures along with bio-fertilizers in T_{16} can be attributed to good growth with these levels of organic manures and bio-fertilizers. The maximum dry leaf yield in T_{16} can be correlated to higher plant height, number of branches and plant spread. Leaf is the only plant part in stevia that contains stevioside and other sweetening compounds and its production was significantly favoured due to the increased levels of manures and bio-fertilizers. Application of organic manures will help in the plant metabolic activity through the supply of required major and micronutrients, such as zinc, iron, copper, manganese (Maheshwarappa *et al.,* 1999). These are involved in biochemical synthesis of many phyto-hormones. Besides, these manures cause better physical, chemical and biological function, which provides carbon as an energy source to soil microbes resulting in enhanced plant growth and yield.

Along with organic manures, application of microbial inoculants *Azospirillum* spp liberate plant growth promoting substances such as gibberellins, cytokinins, indoleacetic acid, vitamins and antibiotics, which suppresses plant pathogens and also help in maintaining soil fertility besides increasing crop yield by 10-15%. Bacterium produces abundant slime, which helps in soil aggregation (Vyas *et al.,* 2008). Similarly, PSB possesses the ability to enhance phosphate solubilization by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, succinic acids *etc.* They also produce plant growth promoting substances like IAA, GA *etc*. (Vyas *et al.*, 2008)

Quality parameters: The highest stevioside (7.8), rebaudiside

Table 5. Influence of different organic manures and bio-fertilizers on stevia (*S. rebaudiana* Bert.) dry leaf yield (kg ha^{-1}) at different harvests during three years of cropping

Treatments	2004-05				I year	2005-06				II year	2006-07			III year
	3 MAP	6 MAP	9 MAP	12 MAP	Total yield	15 MAP	18 MAP	21 MAP	24 MAP	Total yield	27 MAP	30 MAP	33 MAP	Total yield
T_1–FYM15t+VC1.0t+NC0.5t/ha	416.9	552.5	1268.5	827.2	3065.0	577.6	591.7	1049.4	408.0	2026.7	284.4	303.7	341.0	928.8
T_2–FYM15t+VC1.0t+NC0.5t+BF/ha	466.1	688.3	1265.4	879.6	3299.4	714.2	753.1	941.3	187.3	2596.0	270.6	238.9	1100.0	1609.5
T_3–FYM15t+VC1.0t+NC1.0t/ha	382.7	425.9	1345.7	895.1	3049.4	635.2	546.3	864.2	259.3	2305.0	219.1	285.6	877.0	1381.7
T_4–FYM15t+VC1.0t+NC1.0t+BF/ha	592.6	660.5	1731.5	1074.1	4058.7	511.9	462.1	987.6	239.3	2201.0	332.7	242.0	671.0	1243.7
T_5–FYM15t+VC2.0t+NC0.5t/ha	580.2	984.6	1620.4	1043.2	4228.4	543.2	637.3	1018.5	390.3	2589.3	440.9	239.1	801.0	1480.8
T_6–FYM15t+VC2.0t+NC0.5t+BF/ha	589.5	604.9	1271.6	1302.5	3768.6	732.5	625.7	987.6	509.3	2855.1	200.5	283.1	400.0	883.9
T_7–FYM15t+VC2.0t+NC1.0t/ha	530.3	882.7	1577.2	1015.4	4005.6	660.5	436.9	864.2	198.0	2159.6	206.4	164.6	861.0	1231.7
T_8–FYM15t+VC2.0t+NC1.0t+BF/ha	492.3	620.4	1222.2	1006.2	3341.1	1027.2	919.5	1049.4	276.7	3272.7	239.6	192.4	784.0	1215.6
T_9–FYM25t+VC1.0t+NC0.5t/ha	422.8	719.1	1645.1	1104.9	3892.0	952.7	583.3	1203.7	316.3	3056.0	304.3	284.6	325.0	913.6
T_{10}–FYM25t+VC1.0t+NC0.5t+BF/ha	531.5	703.7	2101.9	1277.8	4614.8	614.9	450.6	907.6	307.3	2360.1	177.1	182.1	932.0	1291.5
T_{11}–FYM25t+VC1.0t+NC1.0t/ha	491.7	759.3	2123.5	713.0	4087.4	682.1	571.0	956.8	223.7	2437.5	227.6	228.7	713.0	1169.3
T_{12}–FYM25t+VC1.0t+NC1.0t+BF/ha	562.3	657.4	1197.2	864.2	3281.5	751.9	771.2	1450.6	380.0	3353.7	216.1	263.3	389.0	868.1
T_{13}–FYM25t+VC2.0t+NC0.5t/ha	330.3	916.7	1805.6	996.9	4049.4	905.6	712.5	1111.1	366.7	3095.9	206.5	208.5	281.0	696.4
T_{14}–FYM25t+VC2.0t+NC0.5t+BF/ha	445.4	666.7	1037.0	805.6	2960.8	726.1	577.2	1049.4	288.0	2640.6	239.4	234.5	469.0	943.3
T_{15}–FYM25t+VC2.0t+NC1.0t/ha	441.7	706.8	1956.5	1592.6	4637.9	648.1	546.3	1234.6	303.0	2732.4	165.4	256.5	316.0	738.8
T_{16}–FYM25t+VC2.0t+NC1.0t+BF/ha	525.0	1182.1	2425.9	2030.9	6163.9	1150.6	783.1	1111.1	491.0	3535.8	335.4	270.5	691.0	930.7
T_{17} – Green manuring with horse gram	303.4	799.4	1324.1	1549.4	3976.3	571.0	498.2	1049.4	428.3	2547.0	355.6	200.6	310.0	866.1
T_{18} – Green manuring with cow pea	279.0	577.2	1089.5	753.1	3532.1	779.0	539.1	1142.0	473.0	2933.0	440.2	267.5	634.0	1341.7
S Em±	66.4	198.8	334.3	201.3	472.2	176.4	87.8	118.4	76.1	264.0	191.8	39.5	236.7	244.6
CD (*P*=0.05)	190.5	-	-	577.8	1356.7	-	252.2	-	-	761.2	-	-	-	-

FYM – Farm yard manure; VC – Vermicompost; NC – Neem cake; BF – Bio-fertilizers (*A. brazilense,* PSB-*B. megaterium* and VAM - *G. bagyaraji*) each @ 10 kg ha^{-1}; *MAP – months after planting

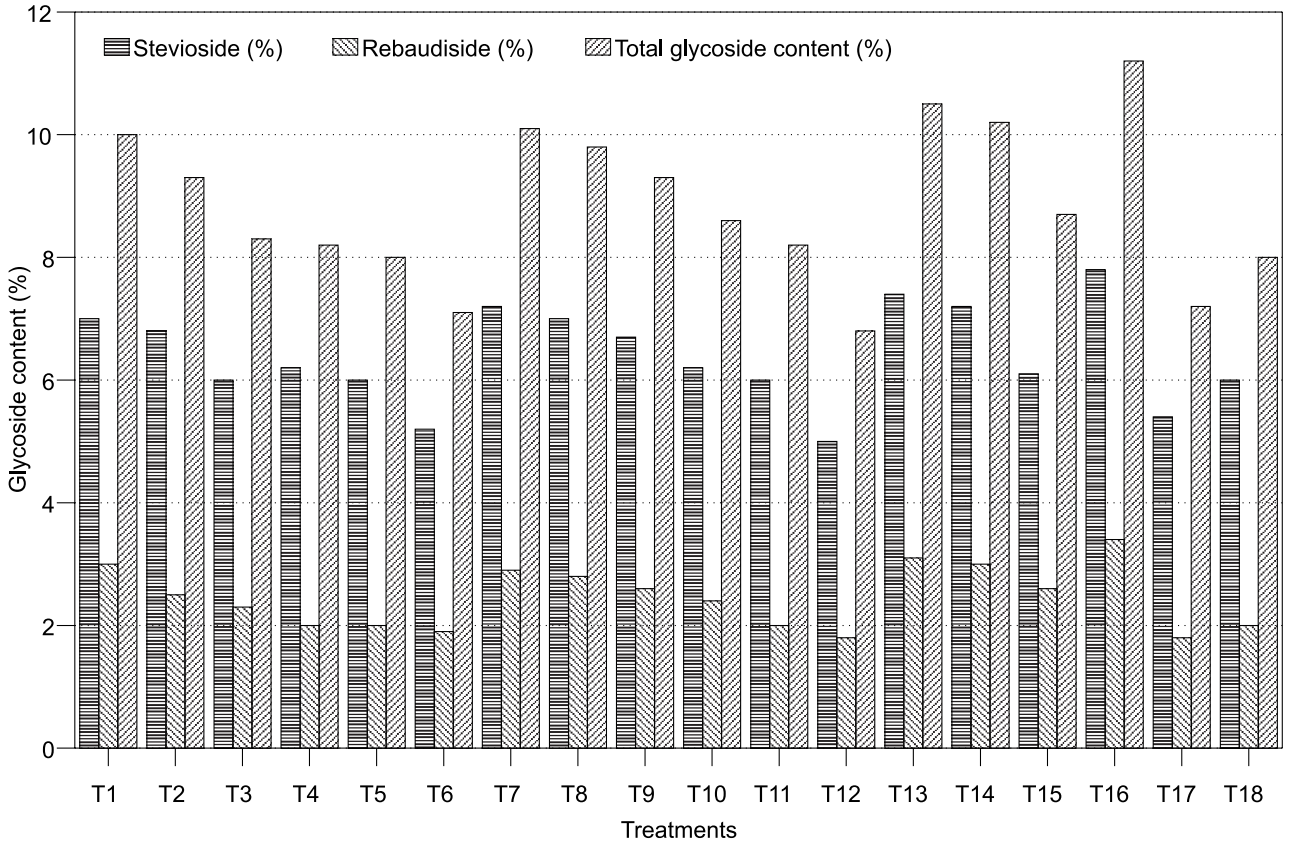

Fig. 1. Influence of different organic manures and bio-fertilizers on glycoside content (% w/w dry weight basis) of stevia leaves (Please refer Table 2 for treatment details)

(3.4) and total glycoside (11.2%) content (w/w) were recorded in the treatment T_{16} (Fig. 1) and the same treatment also had maximum glycoside yield (Table 6) at I and II year (690.36 and 396.01 kg ha^{-1}, respectively). In stevia, quality is a function of secondary metabolites and the secondary metabolites production is always associated with the steady supply of balanced nutrients which was accomplished from the combination of organic manures and bio-fertilizers leading to enhanced quality. The higher glycoside content recorded in the dry stevia leaves in T_{16} may be due to the presence of higher levels of different organic manures and bio-fertilizers. Growth promoting substances like IAA, GA, cytokinin *etc*. secreted by *Azospirillum,* and increased availability of micronutrients due to VAM action might have contributed for the quality improvement. Charan Kumar (2009) in stevia, Nirmalatha *et al.* (2006) in *Andrographis paniculata* and Sudhakara (2005) in *Coleus barbatus* also recorded the same trend.

From the data, it can be inferred that higher levels of organic manures manifested their full potential only when used along with bio-fertilizers. Increased crop yield is a result of better growth, dry leaf yield and glycoside content which ultimately increased the glycoside yield over a period of three years.

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Table 6. Influence of different organic manures and bio-fertilizers on glycoside yield (kg ha^{-1}) of stevia (*S. rebaudiana* Bert.)

Treatments	2004-05	2005-06	2006-07	Pooled mean
T_1–FYM15t+VC1.0t+NC0.5t/ha	306.50	262.67	92.88	220.68
T_2–FYM15t+VC1.0t+NC0.5t+BF/ha	306.84	241.43	149.68	232.65
T_3–FYM15t+VC1.0t+NC1.0t/ha	253.10	191.32	114.68	186.36
T_4–FYM15t+VC1.0t+NC1.0t+BF/ha	332.81	180.48	101.98	205.09
T_5–FYM15t+VC2.0t+NC0.5t/ha	338.27	207.14	118.46	221.30
T_6–FYM15t+VC2.0t+NC0.5t+BF/ha	267.57	202.71	62.76	177.66
T_7–FYM15t+VC2.0t+NC1.0t/ha	404.57	218.12	124.40	249.03
T_8–FYM15t+VC2.0t+NC1.0t+BF/ha	327.43	320.72	119.13	255.76
T_9–FYM25t+VC1.0t+NC0.5t/ha	361.96	284.21	84.96	243.71
T_{10}–FYM25t+VC1.0t+NC0.5t+BF/ha	396.87	202.97	111.07	236.98
T_{11}–FYM25t+VC1.0t+NC1.0t/ha	335.17	199.88	95.88	210.31
T_{12}–FYM25t+VC1.0t+NC1.0t+BF/ha	223.14	228.05	59.03	170.07
T_{13}–FYM25t+VC2.0t+NC0.5t/ha	355.19	325.07	73.12	251.13
T_{14}–FYM25t+VC2.0t+NC0.5t+BF/ha	302.00	269.34	96.22	222.52
T_{15}–FYM25t+VC2.0t+NC1.0t/ha	408.71	237.72	64.28	236.90
T_{16}–FYM25t+VC2.0t+NC1.0t+BF/ha	690.36	396.01	104.24	396.87
T_{17} – Green manuring with horse gram	286.29	183.38	62.36	177.35
T_{18} – Green manuring with cow pea	282.57	234.64	107.34	208.18
SEm±	41.75	26.26	22.34	21.93
LSD (P=0.05)	119.98	75.45	64.20	63.03

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