Comparative Efficiency of Bio- and Chemical Fertilizers on Nutrient Contents and Biomass Yield in Medicinal Plant *Stevia rebaudiana* Bert.

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Received: 30 May 2007
Accepted: 17 July 2007

Abstract

A green house experiment was conducted at the Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bangalore, India to study the comparative effect of agro-techniques on biomass yield and nutrient content in stevia by applied chemical fertilizers (N, P and K as sole and in combinations) and bio-fertilizers (Vesicular Arbuscular *Mycorrhiza*, Phosphorus solubilizing bacteria and *Azospirillum* as sole and in combinations) and the results showed that the biomass yield as well as nutrient content in stevia have been recorded an increase significantly due to inorganic chemical and biofertilizer applications. The magnitude of such increase in biomass yield and nutrient content was recorded significantly higher with the application of biofertilizer, being further greater increase with combined applications of VAM, PSB and *Azospirillum*. The increase of biomass yield was 10.80 and 53.20 % higher with the combined applications of VAM+PSB+*Azospirillum* over that of PSB+VAM and chemical fertilizers (N, P and K), respectively.

Key words: Bio-fertilizer, medicinal plant, NPK content, stevia

INTRODUCTION

Introducing herbal plant stevia as a modern crop from the wild plant increases the recent demand for the high potency sweeteners. Stevia is a South American plant basically from Paraguay and Brazil belonging to the family *Compositae*. Dr. M.S Bertoni officially discovered the sweet herb stevia in early 20th century (1905). Stevia now successfully grown in Paraguay, Mexico, Central America, Japan, China, Malaysia, South Korea and recently started in different parts of India especially in South India. In Europe it is also reported to be cultivated in Spain, Belgium and UK. The first reports of commercial cultivation in Paraguay were in 1964 [1]. Sweetener property and its minimal side effects focused on the importance of stevia, mainly due to the presence of complex mixture of natural sweet diterpene glycosides viz. Stevioside, Rebaudiosides which are 300-350 times and 450 times sweeter than sugar, respectively [2]. Literature survey reveals stevia's versatile applications in treatment of obesity, weight loss, dental health, high blood pressure, oral health, carbohydrate cravings, skin toning and healing, tobacco and alcohol cravings and antihyperglycemic effects of stevioside in type 2 diabetic subjects [3]. The global market size and business of medicinal plant materials including stevia and health care products based on these herbs comes to around 62 billion US $ and is likely to cross the 1 trillion mark by 2020 and 5 trillion by 2050 [4]. These targets only can possible by improving agro-practice with newer techniques.

Several literatures reported that stevioside content varies with the dry weight of the leaves depending on the cultivar and growing conditions [5,6]. Hence, it is necessary to improve agro-techniques for increasing the biomass yield of stevia. Influence of fertilizer levels on growth, yield and uptake of stevia was reported by Chalapathi [7,8], but no such comparative study of agro-technique so far been reported till. Hence, the present investigation was undertaken by separately applied fertilizer and bio-fertilizer on stevia plant.

MATERIALS AND METHODS

Cutting of stevia plants were collected from University of Agricultural Science (UAS), Bangalore, India and were used as a test plant. A green house experiment was conducted during Kharif 2004 at Indian Institute of Horticultural Research, Bangalore under controlled conditions at a temperature of 26 ± 2° C. The location of the experimental site was 12° 58" N latitudes and 77° 33" E longitudes and 930 m above the sea level. Before planting, initial soil pH (soil: water, 1:2.5), organic carbon, CEC, available N, P and K were determined following the method as described by Piper [9] and Jackson [10], respectively. After extracting the soil samples, available N was determined with the help of Kjeldahl Flask Method, available P was determined by spectrophotometer (ECIL, made in India) using red filter at 660 nm and available K was determined by flame photometer (Elico, made in India), respectively. The relevant physicochemical properties of soils were: pH, 8.9; organic carbon, 3.8 $g.kg^{-1}$; available N (NH_4-N: 4.15 $mg.kg^{-1}$; $N0_3$-N; 3.70 $mg.kg^{-1}$), available P (0.56 $mg.kg^{-1}$) and available K (8.70 $mg.kg^{-1}$). Thirty-two earthen pots (15 kgcapacity) were taken and 10 kg-powdered soil collected from the Indian Institute of Horticultural Research Farm, Hessaraghata, Bangalore was filled in each pot and the following treatments were:

For fertilizer experiment: T_1 – absolute control, no application of fertilizers; T_2 – soil application of N at 40 kg.ha^{-1} as urea; T_3 – soil application of P_2O_5 at 20 kg.ha^{-1} single super phosphate; T_4 – soil application of K_2O at 30 kg.ha^{-1} as muriate of potash; T_5 – soil application of N at 40 kg.ha^{-1} as urea and P_2O_5 at 20 kg.ha^{-1} as single super phosphate; T_6 – soil application of P_2O_5 at 20 kg.ha^{-1} as single super phosphate and K_2O at 30 kg.ha^{-1} as muriate of potash; T_7 – soil application of N at 40 kg.ha^{-1} as urea and K_2O at 30 kg.ha^{-1} as muriate of potash; T_8 –soil application of N at 40 kg.ha^{-1} as urea, P_2O_5 at 20 kg.ha^{-1} as single super phosphate and K_2O at 30 kg.ha^{-1} as muriate of potash.

For bio-fertilizer experiment: T_1-absolute control, without application of bio-fertilizers, T_2- soil application of Vesicular Arbuscular Mycorrhiza (VAM) at 25 g, T_3- soil application of Phosphate solubilising bacteria (PSB) at 25 g, T_4- soil application of Azospirillum at 25 g, T_5- soil application in combination of Vesicular Arbuscular Mycorrhiza (VAM) at 25 g, and Phosphate solubilising bacteria (PSB) at 25 g, T_6- soil application in combination of Vesicular Arbuscular Mycorrhiza (VAM) at 25 g, and Azospirillum at 25 g, T_7- soil application in combination of Phosphate solubilising bacteria (PSB) at 25 g and Azospirillum at 25 g, T_8- soil application in combination of Vesicular Arbuscular Mycorrhiza (VAM) at 25 g, Phosphate solubilising bacteria (PSB) at 25 g and Azospirillum at 25 g.

Each treatment of two experiments was replicated four times in a completely randomized design (CRD). There were 32 pots (8×4) altogether for each different experiments. The pots were placed in poly house in order to monitor growth of the plant after cuttings of the Stevia plant were put in each pot. Then the plants were allowed to grow for a period of 60 days. The periodic collection of soil and plant samples was made and analyzed for pH, available N, available P and available K by following the method as mentioned earlier. Besides different yield attributes and yields were also recorded periodically.

RESULTS

Plant sample analysis

Nitrogen content in plant: From the table 1, the results conclude that the N content in plant initially increased up to 45 days of crop growth and thereafter decreased irrespective of treatments. The magnitude of such changes, however, varied with treatments, being highest content (41.22 g.kg^{-1}) followed

Table 1. Effect of mode of application of N, P, K and biofertilizers on the changes in N content (g.kg^{-1}) in plant.

Treatments	15 days		30 days		45 days		60 days	
(T)	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer
T_1	14.12	1.68	19.74	2.80	25.37	2.98	22.60	2.26
T_2	20.72	1.72	28.47	3.18	38.92	3.02	35.30	2.37
T_3	16.88	1.80	22.58	3.51	29.88	3.20	27.10	2.67
T_4	17.10	2.12	23.20	4.40	26.84	3.98	24.30	3.79
T_5	20.42	1.87	29.40	3.64	39.89	3.14	36.28	2.83
T_6	17.22	1.98	24.08	3.98	27.76	3.47	23.78	2.87
T_7	19.94	1.90	27.36	3.72	36.76	3.27	33.46	2.57
T_8	22.37	2.30	32.86	4.75	41.22	4.13	37.80	3.92
C.D. (P 0.05)	0.96	0.26	1.68	0.47	1.84	0.51	1.58	0.59

C.Fer = Chemical Fertilizer

B.Fer = Bio-fertilizer

C.D. = Critical difference

Table 2. Effect of mode of application of N,P,K and biofertilizers on the changes in P content (mg.kg^{-1}) in plant.

Treatments	15 days		30 days		45 days		60 days	
(T)	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer
T_1	2.32	3.42	3.89	5.82	4.98	5.26	4.02	4.82
T_2	2.86	7.90	4.78	14.18	7.36	13.92	6.89	13.38
T_3	4.72	8.50	7.88	16.72	11.37	15.37	9.42	14.48
T_4	3.10	8.39	5.20	15.98	8.96	15.02	7.34	14.10
T_5	4.84	7.82	8.96	14.92	13.48	13.98	9.98	13.40
T_6	4.63	7.88	8.39	14.98	12.98	14.28	10.48	13.92
T_7	3.28	7.80	5.38	15.10	9.87	14.36	9.13	13.71
T_8	4.98	8.42	9.86	16.86	14.77	16.08	11.63	15.52
C.D. (P 0.05)	0.17	0.29	0.92	0.88	1.21	0.79	0.87	0.74

C.Fer = Chemical Fertilizer

B.Fer = Bio-fertilizer

C.D. = Critical difference

Table 3. Effect of mode of applications of N, P, K and biofertilizers on the changes in K content (mg.kg-1) in plant.

Treatments	15 days		30 days		45 days		60 days	
(T)	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer
T_1	238.70	188.92	245.60	262.68	259.74	259.30	251.20	251.20
T_2	239.18	220.89	267.38	396.78	332.14	344.58	320.60	315.65
T_3	237.96	238.92	269.33	405.46	336.80	358.39	334.80	320.72
T_4	268.86	239.69	386.71	403.98	462.35	379.38	448.20	362.48
T_5	239.88	254.28	278.46	423.72	379.72	393.48	364.00	371.32
T_6	272.63	255.10	378.90	424.90	438.82	391.27	410.20	366.44
T_7	266.46	248.78	376.86	408.63	434.94	396.46	412.40	363.46
T_8	291.76	262.42	392.48	488.22	512.16	414.54	508.60	378.20
C.D. (P=0.05)	3.62	3.24	5.72	7.44	18.96	6.86	31.58	5.79

C.Fer = Chemical Fertilizer

B.Fer = Bio-fertilizer

C.D. = Critical difference

by 39.89 g.kg^{-1} in the treatments where recommended levels of N, P and K simultaneously and combined application of N and P, respectively.

Similarly, the results show that the amount of N content initially increased and thereafter, the amount of the same decreased irrespective of treatments due to application of bio-fertilizers. The magnitude of such changes, however, varied with treatments, being highest N content (4.75 g.kg^{-1}) in the treatment where simultaneous applications of VAM, PSB and Azospirillium which was closely followed by the sole application of Azospirillium (4.40 g.kg^{-1}).

P content in plant

The results showed that the changes of P content in the plant almost followed a similar trend to those of N content in the plant. However, the absolute amount of P was recorded as much lower than both N and K contents (Table 2). The amount of P content in plant followed the similar trend to those of N content in plant due to application of bio-fertilizers. The amount of P content, however, recorded highest (16.86 mg.kg^{-1}) in the treatment where VAM, PSB and AZO were applied simultaneously, followed by the treatment (16.72 mg.kg^{-1}) when only PSB was applied.

K content in plant

The results suggested that the amount of K content also followed a similar pattern of changes to those of both N and P contents in plants. The application of recommended levels of N, P and K applied simultaneously showed the highest K content (512.16 mg.kg^{-1}) at 45 days of crop growth which also maintained up to 60 days of crop growth (Table 3). The results show that the amount of K content in plants increased initially at 30 days of plant growth and thereafter, the amount of the same decreased. With the application of bio-fertilizers the amount of the same decreased irrespective of treatments. The highest K content (488.22 mg.kg^{-1}) was recorded in the treatment where VAM+PSB+AZO were applied simultaneously, next followed by VAM+AZO (424.90 mg.kg^{-1}) treatment.

Biomass yield

With regards to the application of bio and chemical fertilizers, the amount of fresh biomass has found to be increased progressively irrespective of treatments in both experiments. However, with chemical fertilization, the magnitude of such increase varied with treatments, being recorded highest (5.39 g) in treatment T_1 (Table 4) at 45 days of plant growth, which was closely followed by 4.76 g in treatment T_8. The total fresh biomass production was also recorded highest 11.84 g after

Table 4. Effect of mode of applications of N, P, K and biofertilizers on the biomass yield (g) of Stevia plant.

Treatments	15 days		30 days		45 days		60 days		Total Biomass	
(T)	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer	C. Fer	B. Fer
T_1	0.80	2.54	1.30	3.58	5.39	3.58	2.60	3.28	10.09	12.57
T_2	1.40	2.32	2.00	3.71	3.35	4.03	2.65	3.92	9.40	14.08
T_3	0.96	2.61	1.36	3.31	4.18	4.82	2.10	3.46	8.60	14.80
T_4	0.91	2.58	1.78	3.32	4.33	5.01	2.34	4.13	9.36	15.04
T_5	0.93	2.31	1.39	3.86	4.19	5.66	2.17	4.55	8.68	16.38
T_6	0.94	2.10	1.29	3.14	4.16	5.39	2.17	4.11	8.56	14.74
T_7	0.90	2.74	1.43	3.20	4.37	5.89	2.25	4.35	8.95	16.18
T_8	1.37	3.01	1.98	4.02	4.76	6.89	3.93	4.23	11.84	18.15
C.D. (P 0.05)	0.48	0.22	0.36	0.26	0.31	0.24	0.27	0.29		

C.Fer = Chemical Fertilizer

B.Fer = Bio-fertilizer

C.D. = Critical difference

60 days of plant growth in treatment T_8 that might be due to simultaneous or combined applications of NPK.

The results (Table 4) show that the amount of fresh biomass yield has found to be increased progressively irrespective of treatments over control. However, the magnitude of such changes varied with treatments, being recorded highest (6.89 g) in treatment T_8 at 45 days of plant growth. The total fresh biomass production was also recorded highest (18.15 g) after 60 days of plant growth in treatment $T_{8.}$

DISCUSSIONS

N Content in Plant

On fertilization nitrogen content in plant initial greater and smaller amount at the later period of crop might be due to less biomass accumulation absorbing very little amount of N and greater biomass production resulting in dilution effect of N absorption by the plant respectively. Shadchina and Dmitrieva [12] found a close relationship between chlorophyll in leaves and accumulation of total nitrogen dry matter of the whole plant. Chlorophyll content is found to be an informative parameter for the estimation of nitrogen uptake from the soil. Chalapathi *et al*. [7] reported that the application of 60:30:45 NPK kg.ha^{-1} produced higher dry leaf yield and recorded higher nutrient uptake by Stevia plant. Sarkar *et al*. [13] reported that the response of N was negative in the absence of phosphorus and grain response with NP fertilization was less than that with NPK fertilizers. Singh and Prasad [14] also reported an increase in nitrogen uptake with an increasing dose of phosphorus resulting from an increased biomass production.

On bio-fertilizer application, increase in N content might be due to VAM, PSB and Azospirillium inoculation together causing relatively greater utilization of available N by plants in presence of VAM and PSB compared to sole inoculation of Azospirillium. However, the decreased amount of N at the later period of crop growth might be due to dilution effect arising from the increased biomass production. Their uses have shown positive interaction with an average dose of applied N in several field crops, with an average response equivalent to 15-20 kgN. ha^{-1} [15]. Pramanik and Singh [16] also reported the similar results who found that PSB+VAM inoculation was superior over PSB or VAM alone with regards to N content and uptake (Table 1).

P content in Plant

With increasing levels of N application up to 75 kgN.ha^{-1}, the contents of N, P and K increase slightly. Phosphorus levels increased N and P contents significantly but did not affect the K content. The N and P uptake increased an increasing level of N up to 75 kgN.ha^{-1}, but that of K uptake reduced at higher levels of N. Increase in P levels increased N, P and K uptake successively. Potassium fertilization improves significantly the utilization of N and P, and stabilizes the yield of wheat [17].

The amount of P content in plant followed the similar trend to those of N content in plant due to application of bio-fertilizers. The results of the present investigation also finds support from the results reported by Pramanik and Singh [16] who showed that the dual or simultaneous inoculation of PSB+VAM was superior with respect to P uptake as compared to sole or separate inoculation of PSB and VAM. The results also pointed out that the amount of P content in plant did not show any significant variation with the combined applications of VAM+AZO, VAM+PSB and PSB+AZO. It is well known that the PSB have ability to solubilise native as well as applied phosphorus. On the other hand, the external hyphae of VAM constitutes an important pathway for phosphate transport through soil as they extend beyond the phosphorus depletion zone surrounding the absorbing root and providing access to phosphorus which otherwise transport only by slow diffusion process [18, 19]. The results also show that the mean percent increase in phosphorus content in stevia plant was recorded highest (193.78 %) with the simultaneous inoculation of VAM+PSB+AZO followed by 184.88 % with the sole inoculation of PSB which also might be explained by the synergistic relationship among themselves resulting greater absorption of phosphorus by stevia plant. Sood and Kumar [11] reported that green and dry foliage yield increased with increasing levels of N and P, the highest yield of 621.22 q ha^{-1} was produced with combined applications of 90 kg N and 75 kg P_2O_5.ha^{-1}. Das [18] reported that the inoculation of VAM helps to increase P uptake from the soil (Table 2).

K content in plant

The results indicated that the combined applications of both P and K; and N and K showed a substantial increase in the amount of K content in plant, but failed to exceed the amount (462.35 mg.kg^{-1}) when only K at 30 kg.ha^{-1} was applied. Sharma and Tandon [20] reported that among major nutrients, high yielding varieties of field and medicinal plants have been found highly responsive to nitrogen fertilization. However, in the absence of phosphorus, nitrogen becomes ineffective and most of the applied nitrogen remains unutilized.

The results clearly pointed out that the sole application of VAM, PSB and AZO has not been proven superior with regards to K content in plant compared to their combined applications (Table 3). Inoculation with single inoculum, Azospirillum results in enhanced assimilation of mineral nutrients especially K in plants, but such assimilation of K in plants might be further enhanced with their dual (PSB+AZO; VAM+PSB; VAM+AZO) or triple inoculation (VAM+PSB+AZO) resulting from their strong synergistic relationships [16, 19].

Biomass yields

Results showed height biomass yielding of 18.15 g, which might be explained due to their ability to fix atmospheric nitrogen either symbiotically or non-symbiotically and hence transform native soil nutrients such as phosphorus, zinc, iron, copper sulfur from insoluble to soluble forms which can easily be assimilated by plants resulting an increase in biomass yield and nutrient content in the stevia plant.

CONCLUSIONS

The application of inorganic chemical fertilizer as a single nutrient source i.e. sole application of N/P/K enhanced the biomass yield and nutrient content in stevia plant over that of absolute control, no application of fertilizers, but such increase in biomass yield and nutrient content has been found to be further enhanced due to simultaneous applications of N, P and K fertilizers. The results further suggested that the biomass yield and nutrient content in stevia plant has been markedly increased due to bio-fertilizer application compared to inorganic chemical fertilizers either as single or combined applications. However, the overall results suggested that the combined application of bio-fertilizers was always superior as compared to combined or simultaneous applications of inorganic chemical fertilizers N, P and K.

ACKNOWLEDGEMENTS

The authors are grateful to the Indian Institute of Horticultural Research, Bangalore, India, for rendering help and co-operation in analyzing soil and plant samples during the course of the present investigation as a part of postgraduate dissertation.

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