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Full Length Research Paper

Nutrient Composition in (*Stevia rebaudiana)* Bert. grown in Indian sub-tropics and influence of bio- fertilizers on the biomass yield

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A pot culture experiment was conducted at Indian Institute of Horticultural Research, Hessaraghata, Bangalore, India to study the effect of bio-fertilizers on the biomass yield and NPK content in Stevia (*Stevia rebaudiana*). The results show the yield and NPK content in stevia plant has been found to be increased initially and thereafter, the amount of the same decreased with the progress of plant growth up to 60 days with the combined treatment of bio-fertilizers rather than individual treatment. This is due to their ability to fix atmospheric nitrogen (symbiotic and asymbiotic) and transform native soil nutrients likely phosphorus, zinc, copper, iron, sulfur from the non -usable (fixed) to usable form and decompose organic wastes through biological processes which in turn releases nutrients in a form which can be easily assimilated by plants resulting in an increase in biomass production of stevia plant.

Key words: Azospirillum (AZO), bio-fertilizer, phosphorus solubilising bacteria (PSB), *Stevia rebaudiana,* vesicular arbuscular mycorrhiza (VAM)

INTRODUCTION

Stevia rebaudiana (Bert), officially discovered by Dr. M.S. Bertoni in 1905, belonging to the family *Compositae* is a recent high demanding secondary metabolite in herbal world. Health causing diseases by natural caloric sweetener as well as by chemical sweeteners (like saccharin and aspartame) make the life risk. So the focus came to on stevia which is completely natural and non-caloric plant. The plant is native to South America (Paraguay and Brazil) but recently domesticated in India through its cultivation.

The fresh leaves itself 30 - 45 times (Mowrey, 1992) and the main active principal stevioside is 200 - 300 times (Bridel and Lavielle, 1931) sweeter than sugar.

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Abbreviations: VAM, Vesicular Arbuscular Mycorrhiza; PSB, Phosphorus S olubilising bacteria; AZO, Azospirillum.

Stevioside concentrations usually range from 3 - 10% of leaf dry weight, rebaudioside-A (400 times sweeter than sugar) is less concentrated ranging from 1 - 3% (Cramer and Ikan, 1986). The amounts of active principles are depending on total biomass yield which is further depends on the climatic feature, method of agrotechniques, water management and also fertilizer applications. So yield improvement can be achieved by standardizing the agronomy especially with respect to those parameters. Stevia (*Stevia rebaudiana*) plant has a high requirement of nutrients especially N, P and K and their deficiency is probably the major limitation for the quality biomass production of stevia (Pramanik and Singh, 2003). There is a need to develop a suitable agricultural system which requires lower fertilizer input with higher fertilizer use efficiency. Recently, Vesicular Arbuscular Mycorrhiza (VAM), Phosphate Solubilizing Bacteria (PSB) and Azo-spirillum (AZO) are the most widespread bio-fertilizers significantly contributing N, P and K to plants and also providing resistance to drought situation (Smith and Read

Table 1. Effect of bio-fertilizer inoculation on the biomass yield (g) of Stevia plant.

Treatments	15 days	30 days	45 days	60 days	Total biomass (g)	Response of biomass yield (%)
Control	2.54	3.58	3.58	3.28	12.57	-
VAM (V)	2.32	3.71	4.03	3.92	14.08	12.01
PSB (P)	2.61	3.31	4.82	3.46	14.80	17.74
AZO (A)	2.58	3.32	5.01	4.13	15.04	19.64
VAM+PSB	2.31	3.86	5.66	4.55	16.38	30.31
VAM+AZO	2.10	3.14	5.39	4.11	14.74	17.26
PSB+AZO	2.74	3.20	5.89	4.35	16.18	28.71
VAM+PSB+AZO	3.01	4.02	6.89	4.23	18.15	44.39
CD (P= 0.05)	0.22	0.26	0.24	0.29	0.32	

1997). However, very limited or no information is available regarding the effect of bio-fertilizers on the yield and major nutrient contents in stevia. In view of the above facts, the present investigation was undertaken to recommend improved technology of bio-fertilizer application on the yield and nutrient content in stevia plant.

MATERIALS AND METHODS

Stem cuttings of stevia plant were collected from Gandhi Krishi Vigyan Kendra, Bangalore and were used as test plants. Before planting, initial soil samples were analyzed for pH (soil: water, 1:2.5), organic carbon, available N, P and K from soil by following the methods as described by Jackson (1973). After extracting the soil samples available N was determined by titrating against standard 0.01 (N) H_2S0_4, available phosphorus by spectrophotometer (ECIL, made in India) and available K by using flame photometer (Elico, made in India). 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 numbers of earthen pot having capacity of 15 kg soil were taken and ten (10) kg powdered soil collected from IIHR, Hessaraghatta, Bangalore was filled up to each pot and the following treatments were, T_1: absolute control, without application of bio-fertilizers; T_2: soil application of VAM- 25 g; T_3: soil application of PSB-25 g; T_4: soil application of AZO-25 g; T_5: soil application in combination of VAM-25 g and PSB-25 g; T_6: soil application in combination of VAM-25 g and AZO-25 g; T_7: soil application in combination of PSB-25 g and AZO-25 g; T_8: soil application in combination of VAM-25 g, PSB-25 g and AZO-25 g.

Each treatment was replicated four times in a completely randomized design (CRD). There was altogether (8x4) = 32 pots. The pots were placed in green house for monitoring growth of the plant after putting cuttings of the stevia plant in each pot. Then the plants were allowed to grow for a period of 60 days. The periodical collection with 15 days intervals of soil and plant samples were made and analyzed for pH, available N, P and K by following the methods as mentioned earlier. Besides, different yield attributes and yields were also recorded periodically.

RESULTS AND DISCUSSION

Biomass yield: The results (Table 1) show that the amount of fresh biomass yield has been 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 bio-mass production was also recorded highest (18.15 g) aft-er 60 days of plant growth in the treatment T_8 which might be due to combined application of bio-fertilizers which caused maximum fixation of atmospheric nitrogen, inc-reased uptake of soil phosphorus and potassium by the stevia plant as compared to their respective sole appli-cations. The total biomass yield was followed in the order of VAM+PSB+AZO > VAM+PSB > PSB+AZO > AZO > PSB > VAM+AZO > VAM > Control. The percent respon-se of microbial inoculant towards the total biomass yield was recorded highest (44.39%) in the treatment where VAM+PSB+AZO was inoculated altogether which was closely followed by VAM+PSB (30.31%) and PSB+AZO (28.71 %). Chalapathi et al. (1997, 1999) reported that the application of N, P and K at 60, 30 and 45 kg ha^{-1}, respectively, produce higher dry leaf yield with simultan-eous higher nutrient content and uptake by stevia plant.

Nitrogen content in plant: The results (Table 2) showed that the amount of N content initially increased and thereafter, the amount of the same decreased irrespective of treatments. 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 AZO which was closely followed by the sole application of AZO (4.40 g kg^{-1}). Such increase in N content might be due to VAM, PSB and AZO inoculation together causing relatively greater utilization of available N by plants in presence of VAM and PSB compared to sole inoculation of AZO. 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. The mean percent increase of N content in plant over control was recorded highest (55.14%) in the treatment simultaneously inoculating with VAM+PSB+AZO followed by 46.91% in the sole inoculation of AZO. Azospirillum species was found to be especially effective on N-fixation in low land rice. Their use was shown positive interaction with an average dose of applied N in several field crops, with an average response equivalent to 15-20

Table 2. Effect of bio-fertilizer inoculation on the nitrogen content (g kg^{-1}) in Stevia plant.

Treatments	15 days	30 days	45 days	60 days	Total biomass (g)	Mean increase over control (%)
Control	1.68	2.80	2.98	2.26	2.43	
VAM (V)	1.72	3.18	3.02	2.37	2.57	5.76
PSB (P)	1.80	3.51	3.20	2.67	2.79	14.81
AZO (A)	2.12	4.40	3.98	3.79	3.57	46.91
VAM+PSB	1.87	3.64	3.14	2.83	2.87	18.10
VAM+AZO	1.98	3.98	3.47	2.87	3.07	26.33
PSB+AZO	1.90	3.72	3.27	2.57	2.86	17.69
VAM+PSB+AZO	2.30	4.75	4.13	3.92	3.77	55.14
CD (P= 0.05)	0.26	0.47	0.51	0.59		

Table 3. Effect of bio-fertilizer inoculation on the phosphorus content (mg kg^{-1}) in Stevia plant.

Treatments	15 days	30 days	45 days	60 days	Total biomass (g)	Mean increase over control (%)
Control	3.42	5.82	5.26	4.82	4.83	
VAM (V)	7.90	14.18	13.92	13.38	12.34	155.48
PSB (P)	8.50	16.72	15.37	14.48	13.76	184.88
AZO (A)	8.39	15.98	15.02	14.10	13.37	176.81
VAM+PSB	7.82	14.92	13.98	13.40	12.53	159.42
VAM+AZO	7.88	14.98	14.28	13.92	12.76	164.18
PSB+AZO	7.80	15.10	14.36	13.71	12.24	153.41
VAM+PSB+AZO	8.42	16.86	16.08	15.52	14.19	193.78
CD (P= 0.05)	0.29	0.88	0.79	0.74		

kgN ha^{-1} (Biswas et al., 2001) . Pramanik and Singh (2003) also reported the similar results who found that PSB+VAM inoculation was superior over PSB or VAM alone with regards to N content in and uptake by plants.

Phosphorus content in plant: The results (Table 3) showed that the amount of P content in plant followed the similar trend to those of N content in plant. The amount of P content was, 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}) where only PSB was inoculated. Such highest content of P in stevia plant might be due to synergistic effect among VAM, PSB and AZO. The results of the present investigation also finds support from the results reported by Pramanik and Singh (2003) who showed that the dual inoculation of PSB+VAM was superior regarding P uptake as compared to sole 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 dual inoculation of VAM+AZO, VAM+PSB and PSB + AZO. It is well known that the PSB have ability to solubilize 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 phosphorrus which otherwise transport only by slow diffusion process (Das, 2004; Pany, 2003) . The results also show that the mean percent increase in phosphorus content in ste-via plant was recorded highest (193.78%) with the simul-taneous inoculation of VAM + PSB + AZO followed by 184.88% with the sole inoculation of PSB which might be explained by the synergistic relationship among themsel-ves resulting in greater absorption of phosphorus by ste-via plant. Das (2004) reported that the inoculation of VAM helps to increase P uptake from the soil.

Potassium content in plant: The results (Table 4) showed that the amount of K content in plants increased initially (30 days) and thereafter, the amount of the same decreased with the progress of plant growth irrespective of treatments. The highest K content (488.22 mg kg^{-1}) was recorded in the treatment where VAM+PSB+AZO were inoculated simultaneously, next followed by VAM + AZO (424.90 mg kg^{-1}) treatment. The results clearly pointed out that the sole inoculation of VAM, PSB and AZO has not been proved superior with regards to K content in stevia plant compared to their combined inoculations. The results further show that the percent increase of K content in stevia plant was recorded highest (60.41%) in the treatment where VAM + PSB + AZO was inoculated togetherly. 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;

Table 4. Effect of bio-fertilizer inoculation on the potassium content (mg kg^{-1}) in Stevia plant

Treatments	15 days	30 days	45 days	60 days	Total biomass (g)	Mean increase over control (%)
Control	188.92	262.68	259.30	251.20	240.52	
VAM (V)	220.89	396.78	344.58	315.65	319.47	32.82
PSB (P)	238.92	405.46	358.39	320.72	330.87	37.56
AZO (A)	239.69	403.98	379.38	362.48	346.38	44.01
VAM+PSB	254.28	423.72	393.48	371.32	360.70	49.96
VAM+AZO	255.10	424.90	391.27	366.44	359.42	49.43
PSB+AZO	248.78	408.63	396.46	363.46	354.33	47.31
VAM+PSB+AZO	262.42	488.22	414.54	378.20	385.84	60.41
CD (P= 0.05)	3.24	7.74	6.86	5.79		

VAM+PSB; VAM+AZO) or triple inoculation (VAM + PSB + AZO) resulting from their strong synergistic relationships (Pramanik and Singh, 2003; Pany, 2003).

Conclusions

S. rebaudiana Bert. is a unique medicinal plant which is mostly utilized as a sugar substitute for diabetic patients. For the maintenance of its medicinal value, integrated and balanced nutrient management is necessary without deteriorating the quality. Bio- fertilizers are eco friendly and are environmentally safe. They form not only part of integrated nutrients but are of low cost which is of immense help to the farming community. Bio-fertilizers are widely used in crop production. Therefore, the application of bio-fertilizers in their combinations was undertaken in the present study. The results show that an inoculation of a single bio-fertilizer significantly increased the biomass yield as well as nutrient content in plants. However, such increased effects have been found to be further enhanced significantly due to dual or other compatible mixtures of inoculants resulting from their strong synergistic relationships among themselves.

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