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Effect of spacing and bio fertilizers on yield and quality parameters of stevia (*Stevia rebaudiana* Bertoni)

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ABSTRACT

A field experiment on efect of spacing and bio fertilizers on growth and yield of stevia (*Stevia rebuadiana* Bertoni) was conducted at Department of Horticulture, Ch. Chhotu Ram P.G College, Muzaffaranagar (Uttar Pradesh) during *Kharif* seasons of 2006-07 and 2007-08. The experiment was laid out in split plot design with main plot having four spacing levels (30 cm x 20 cm, 30 cm x 30 cm, 45 cm x 20 cm and 45 cm x 30 cm) and sub-plot treatments included six biofertlizer based nutritional treatments [100% NPK (Recommended dose: 60:30:45 kg/ha), 75% N + 100% PK + *Azotobactor*, 100% N + 100% PK + *Azotobactor*, 75% N + 100% PK + *Azospirillum*, 100% N + 100% PK + *Azospirillum* and Control (no fertilizer)] with three replications and 24 treatment combinations. The spacing of S_1 30 x 20 cm and S_2 30 x 30 cm recorded significantly higher herb yields and were at par with fresh (36.53 and 33.68 t/ha) and dry (8.02 and 8.32 t/ha) herb yield, respectively. The fresh (20.10 t/ha) (S_1) and dry (4.58 t/ha) (S_2) stem yield and fresh (16.43 t/ha) (S_1) and dry (3.73 t/ha) (S_2) leaf yield where higher as compared to 45 x 20 cm and 45 x 30 cm spackings. Significantly higher stevioside content (8.06%) was recorded in the spacing of 30 cm x 30 cm than other spacing levels. Significantly lower stevioside content (4.13%) was recorded in the spacing of 45 cm x 30 cm. Among biofertilizer treatments, significantly higher fresh and dry herb yield per hectare was recorded in the treatment that received 100% N + 100% PK+*Azotobactor* (35.72 and 8.30 t/ha, respectively), fresh and dry stem yield per hectare (19.63 and 4.56 t/ha, respectively) and significantly higher fresh and dry leaf yield (16.09 and 3.73t/ha , respectively) as compared to other treatment combinations.

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Key words : *Stevia rebaudiana,* Spacing, Stevioside, Bio-fertilizers

INTRODUCTION

Stevia rebaudiana (Bertoni) was officially discovered by Dr. M. S. Bertoni in 1905, belongs to the family Compositae, is a recent high demand medicinal crop in herbal world. Foods that may cause diseases by the use of natural caloric sweetener as well as by chemical sweeteners (like saccharin and aspartame) make the life risky especially for the middle aged, diabetic and other susceptible groups. So the focus came on stevia which is completely natural and zero-calorie plant. The plant is native to South America (Paraguay and Brazil) but recently domesticated in India for its large scale cultivation. Above all, recently *Stevia rebaudiana* is gaining momentum due to its novel natural sweetener properties and as an alternative sweetener source for the diabetic people. Looking to its present and prospective domestic and global demand for a variety of alleged medicinal effects, the availability of quality raw materials of this wonder plant is very meagre. Since, it is newly adopted crop; there is almost no information available on its proper production techniques which may be one of the causes for the non-availability of its quality raw materials. The modern and intensive agricultural methods are not only costly, but also cause soil and water pollution along with diminishing the quality of the raw materials. Thus, in this situation, the recent concept of ecofriendly technology, application of bio-fertilizers in combination with inorganic fertilizers substitutes may prove to be necessary for this potential crop. In stevia, leaves are the economic part of the plant. The agronomic manipulations and practices aimed at improving the yield of leaves through optimizing source-sink ratio are of more practical significance. Optimum spacing provided to each plant helps to utilize growth resources optimally resulting in better yields. Hence, keeping in view the above facts the present investigation entitled Effect of spacing and bio -fertilizers on growth and yield of stevia (*Stevia rebaudiana* Bertoni)

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was planned to find out the influence of different plant densities and biofertilizers on growth and yield.

MATERIALS AND METHODS

The present study on effect of spacing and biofertilizers on growth and yield of stevia (*Stevia rebaudiana* Bertoni) was conducted during the year 2006 - 2008. The experimental site is situated at 29^0 28^l N, longitude of 77^0 44^l, East and at an altitude of 245.82 meters above mean sea level. The topography of the experimental site was uniform with adequate irrigation and drainage facilities. The experiment was laid out in split plot design with three replications and 24 treatment combinations. The treatments in each plot were allotted randomly and the details of treatments are as follows.

Details of treatments:

Main treatments (Spacing):

S_1 = 30x20 cm
S_2 = 30x30 cm
S_3 = 45x20 cm
S_4 = 45x30 cm

Sub treatments (Nutrients):

F_1= 100%NPK (Recommended dose-60:30:45 kg/ha)
F_2= 75% N+100%PK+*Azotobacter*
F_3= 100% N+100%PK+*Azotobacter*
F_4 = 75%N+100%PK+*Azospirillum*
F_5= 100%N+100%PK+*Azosprillum*
F_6= Control (No fertilizer)

Treatment combinations:

S_1F_1= 30x20 (cm) spacing with 100% NPK
S_1F_2= 30x20 (cm) spacing with 75% N+100%PK+ *Azotobactor*
S_1F_3= 30x20 (cm) spacing with 100% N+100%PK+ *Azotobactor*
S_1F_4=30x20 (cm) spacing with 75% N+100%PK+ *Azospirillum*
S_1F_5= 30x20 (cm) spacing with 100% N+100%PK + *Azospirillum*
S_1F_6=30x20 (cm) spacing with no fertilizer (Control)
S_2F_1= 30x30 (cm) spacing with 100 % NPK
S_2F_2=30x30 (cm) spacing with 75% N+100%PK+ *Azotobactor*
S_2F_3=30x30 (cm) spacing with 100 % N +100%PK+ *Azotobactor*
S_2F_4=30x30 (cm) spacing with 75 % N+100%PK+ *Azospirillum*
S_2F_5=30x30 (cm) spacing with 100 % N +100%PK+ *Azospirillum*
S_2F_6=30x30 (cm) spacing with no fertilizer (Control)
S_3F_1= 45x20 (cm) spacing with 100% NPK
S_3F_2= 45x20 (cm) spacing with 75% N+100%PK+ *Azotobactor*
S_3F_3= 45x20 (cm) spacing with 100 % N+100%PK+ *Azotobactor*
S_3F_4= 45x20 (cm) spacing with 75 % N +100%PK+ *Azospirillum*
S_3F_5= 45x20 (cm) spacing with 100% N +100%PK+ *Azospirillum*
S_3F_6= 45x20 (cm) spacing with no fertilizer (Control)
S_4F_1= 45x20 (cm) spacing with 100% NPK
S_4F_2= 45x20 (cm) spacing with 75%N+100%PK+ *Azotobactor*
S_4F_3= 45x20 (cm) spacing with 100% N+100%PK+ *Azotobactor*
S_4F_4= 45x20 (cm) spacing with 75% N+100%PK+ *Azospirillum*
S_4F_5= 45x20 (cm) spacing with100% N+100%PK+ *Azospirillum*
S_4F_6= 45x20 (cm) spacing with no fertilizer (control)

The nutrients were applied in the form of straight fertilizers *i.e.* nitrogen in the form of urea, phosphorus in the form of single super phosphate and potassium in the form of muriate of potash. Thirty days old healthy and uniform rooted cuttings of stevia were planted as per design and treatments in their respective plots. Before planting, the rooted cuttings were treated well with bioinoculum of *Azotobactor and Azospirillum* in the month of July 2006 and August 2007. The gap filling was taken up within 15 days, wherever it was necessary. The recommended dose of 60 kg nitrogen, 30 kg phosphorus and 45 kg potassium per hectare was applied as per the treatments. Full dose of phosphorus and potassium along with one fifth of nitrogen were applied as basal dose and the remaining nitrogen was given in four split doses as top dressing after 15 days of each harvesting. The crop was harvested at the stage of pre flowering stage at 60-90 days interval by cutting at ground level and dried under shade for 2-5 days before storage and processing. Observation on growth and yield parameters of stevia were recorded at 30 days of planting and the data was analyzed using standard procedures.

RESULTS AND DISCUSSION

The spacing of 30x20cm and 30x30cm recorded significantly higher fresh and dry (16.43t/ha and 3.59t/ha) and (15.33.t/ha and 3.73t/ha) leaves yield per hectare,

Table 1 : Effect of spacing and biofertilizers on fresh and dry leaves yield per ha in stevia

Treatments	Fresh leaves yield /ha (t)					Dry leaves yield /ha (t) (kg)				
Main / Sub	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	16.41	14.90	15.01	8.05	13.59	3.55	3.62	2.94	1.89	3.00
F_2	16.57	15.27	15.11	8.17	13.78	3.59	3.71	3.46	1.92	3.17
F_3	19.32	19.01	17.12	8.93	16.09	4.25	4.65	3.94	2.09	3.73
F_4	16.96	15.92	16.34	8.74	14.49	3.73	3.90	3.79	2.05	3.37
F_5	17.23	18.71	16.44	8.85	15.31	3.79	4.58	3.78	2.08	3.56
F_6	12.08	8.18	8.42	5.70	8.60	2.66	1.93	1.93	1.34	1.96
Mean	16.43	15.33	14.74	8.07		3.59	3.73	3.31	1.89	
C.D. (P=0.05)	0.77					0.285				
B	0.69					0.213				
AxB	1.48					0.480				

S_1= 30x20 cm
S_2= 30x30 cm
S_3= 45x20 cm
S_4= 45x30 cm
* : Significant
NS=Non-significant

F_1= 100%NPK (Recommended dose-60:30:45 kg/ha)
F_2= 75% N+PK+Azoto
F_3= 100% N+PK+Azoto
F_4 = 75%N+PK+Azosp
F_5= 100%N+PK+Azosp
F_6= Control(no Fertilizer)

respectively as compared to other spacing levels. Significantly lower fresh (8.07t/ha) and dry (1.89t/ha) leaf yield was recorded at a spacing of 45x30cm (Table 1).The lower spacing (30x20cm) gave comparatively higher fresh leaf yield but more dry leaf yield was found in 30x30cm which may be due to more metabolites accumulation resulted by sufficient light penetration and maximum photosynthetic activities and hence higher wet to dry ratio. These results are in conformity with the findings of Aiello and Clementel (1987) in salvia.

With the application of 100% N + 100% PK + *Azotobactor* and 100% N + 100% PK + *Azospirillum* the fresh and dry leaf yield also increased at all the stages. Significantly higher fresh and dry leaf yield (16.09t/ha and 3.73t/ha, respectively) was recorded with application of 100% N + 100% PK + *Azotobactor* where as lower fresh and dry leaf yield (8.60t/ha and 1.96t/ha, respectively) was recorded with no application treatment (Table 1). The increase in leaf yield resulted in significant biomass accumulation in top portion, which could be attributed to differences in growth parameters such as plant height and its spread. Similar results were reported by Manjunatha (2000) in patchouli.

The spacing of 30x20cm and 30x30cm recorded

Table 2 : Effect of spacing and biofertilizers on fresh and dry stem yield per ha in stevia

Treatments	Fresh stem yield /ha (t)					Dry stem yield /ha (t) (kg)				
Main / Sub	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	20.147	18.280	18.430	9.990	16.712	4.430	4.480	4.240	2.350	3.875
F_2	19.923	18.740	18.547	10.213	16.856	4.380	4.590	4.263	2.397	3.908
F_3	23.717	23.113	20.710	10.967	19.627	5.217	5.660	4.763	2.580	4.555
F_4	20.823	19.707	20.097	10.780	17.852	4.577	4.827	4.623	2.537	4.141
F_5	21.150	22.210	20.080	10.647	18.522	4.650	5.443	4.617	2.503	4.303
F_6	14.837	10.040	10.303	7.043	10.556	3.267	2.460	2.373	1.653	2.438
Mean	20.099	18.682	18.028	9.940		4.420	4.577	4.147	20.337	
C.D. (P=0.05)	0.958					0.212				
B	0.882					0.197				
AxB	1.868					0.416				

S_1= 30x20 cm
S_2= 30x30 cm
S_3= 45x20 cm
S_4= 45x30 cm
* : Significant
NS=Non-significant

F_1= 100%NPK (Recommended dose-60:30:45 kg/ha)
F_2= 75% N+PK+Azoto
F_3= 100% N+PK+Azoto
F_4 = 75%N+PK+Azosp
F_5= 100%N+PK+Azosp
F_6= Control(no Fertilizer)

significantly higher fresh and dry (20.09 t/ha and 4.42 t/ha) and (18.68t/ha and 4.58t/ha, respectively) stem yield per hectare respectively as compared to other spacing levels. Significantly lower fresh and dry (9.94t/ha, 2,34 t/ha) stem yield was recorded in the spacing of 45x30cm (Table 2). This may be due to wider spacing levels, wider interspaces between plants which could have provided congenial environment for producing more number of branches and leaves owing to improved sunlight interception by plants. These results are in conformity with the findings of Aiello and Clementel (1987) in salvia.

Higher fresh and dry stem yield per hectare was recorded in treatment combination that received 100%N + 100%PK +*Azatobactor* (19.63 and 4.56t/ha), respectively, compared to other bio fertilizer treatments and control. This might be due to the fact that *Azotobactor* produces a variety of growth promoting substances like indole acetic acid (IAA), gibberellins (GA), vitamin-B and antifungal substances. The findings of this study are in tune with those of Omer *et al.* (1992) in *Silybum marianum* and Johri *et al.* (1991) in chamomile.

The herb yield of stevia differed significantly due to spacing levels (30x20 cm, 30x30 cm, 45x20cm and 45x30cm). The spacing of 30x20cm and 30x30cm recorded significantly higher fresh (36.53 and 33.68 t/ha) and dry herb yield (8.02 and 8.32 t/ha), respectively than the other spacing levels (Table 3). Biological yields become asymptotic with increase in plant density .The findings of the present investigation are in line with those of Vadeil *et al.* (1980) in *Mentha citrata,* Gill and Sharma (1986) in fennel and Singh *et al.* (1983) in coriander.

Significant herb yield differences were observed among different biofertilizer levels. The mean data indicated that application of 100% N +100% PK + *Azotobactor* and 100% N + 100%PK + *Azospirillum* recorded significantly higher fresh and dry (35.72t/ha, 8.30 t/ha) and (33.83t/ha and 7.87t/ha) herb yield respectively, than control (18.60 t/ha and 4.41 t/ha) (Table 3). The results are in conformity with the findings of Pillai and Bhoominathan (1975) in coriander.

In the present investigation, significantly higher stevioside content was observed in the treatment with the application of 100% N+100%PK+*Azotobactor* (Table 4). Stevioside content (6.89%) was higher in the treatment that received 100% N + 100%PK + *Azotobacter* as compared to other treatments. Significantly lower stevioside content (4.55%) was recorded in control. The findings are in conformity with the findings of Maheshwari *et al.* (1991) in field experiment on palmarosa (*Cymbopogan martini* Stapf, Var. *motia*) under irrigated conditions to find out the influence of *Azotobactor* and nitrogen on growth of palmarosa.

Interaction effects of spacing and bio fertilizers significantly influenced the stevioside content. Significantly higher stevioside content (9.96%) was observed in the treatment combinations 30x30 cm with biofertilizer level of 100%N + 100%PK + *Azotobactor* than other treatment combinations, higher stevioside content in the above treatment combinations could be due to higher herbage yield.

Table 3 : Effect of spacing and biofertilizers on fresh and dry herb yield per ha in stevia

Treatments	Fresh herb yield /ha (t)					Dry herb yield /ha (t) (kg)				
Main / Sub	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	36.560	33.183	33.447	18.047	30.309	7.990	8.107	7.187	4.240	6.881
F_2	36.500	34.010	33.650	24.557	32.179	7.977	8.307	7.727	4.323	7.083
F_3	43.040	42.123	37.833	19.900	35.724	9.470	10.323	8.703	4.683	8.295
F_4	37.787	35.840	36.443	19.533	32.401	8.310	8.730	8.420	4.590	7.513
F_5	38.383	40.927	36.523	19.500	33.833	8.447	10.027	8.400	4.587	7.865
F_6	26.923	16.003	18.730	12.747	18.601	5.923	4.397	4.307	2.993	4.405
Mean	36.532	33.681	32.771	19.047		8.019	8.315	7.457	4.236	
C.D. (P=0.05)	2.928					0.479				
B	2.510					0.374				
AxB	5.420					0.830				

S_1= 30x20 cm
S_2= 30x30 cm
S_3= 45x20 cm
S_4= 45x30 cm
* : Significant
NS=Non-significant

F_1= 100%NPK (Recommended dose-60:30:45 kg/ha)
F_2= 75% N+PK+Azoto
F_3= 100% N+PK+Azoto
F_4 = 75%N+PK+Azosp
F_5= 100%N+PK+Azosp
F_6= Control(no Fertilizer)

Table 4 : Effect of spacing and biofertilizers on stevioside content (%) in stevia

Treatments	Stevioside				
Main / Sub	S_1	S_2	S_3	S_4	Mean
F_1	5.500	7.250	6.930	4.050	5.933
F_2	5.750	7.803	6.950	4.090	6.148
F_3	6.190	9.960	7.220	4.177	6.887
F_4	5.280	8.900	7.650	3.943	6.443
F_5	4.870	9.080	7.493	4.417	6.465
F_6	4.680	5.340	4.100	4.073	4.548
Mean	5.378	8.056	6.724	4.125	
C.D. (P=0.05)	0.3179				
B	0.2050				
AxB	0.4890				

S_1= 30x20 cm
S_2= 30x30 cm
S_3= 45x20 cm
S_4= 45x30 cm
* : Significant
NS=Non-significant

F_1= 100%NPK (Recommended dose-60:30:45 kg/ha)
F_2= 75% N+PK+Azoto
F_3= 100% N+PK+Azoto
F_4 = 75%N+PK+Azosp
F_5= 100%N+PK+Azosp
F_6= Control(no Fertilizer)

Conclusion:

Higher fresh and dry herb yield was realized at closer spacing (30x20cm and 30x30cm) on per hectare basis. Though stevia produce comparatively low biomass at closer spacings, the total yields per unit area where higher than the plants cultivated with wider spacings. This indicates that the lower yields are compensated by the higher number of plants per unit area at lower spacings. Among the nutrient treatments, application of 100% N + 100%PK + *Azotobacter* and 100% N + 100%PK + *Azospirillum* recorded significantly higher dry herb yield and stevioside content than control.

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