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Effect of different levels of farmyard manure and nitrogen on the yield and nitrogen uptake by stevia (*Stevia rebaudiana* Bertoni)

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Two independent field experiments were conducted at Punjab Agricultural University, Ludhiana, Punjab, India in 2006 and 2007 in a loamy sand soil normal in soil reaction and electrical conductivity, low in organic carbon and available nitrogen, medium in phosphorus and potassium status in a complete randomized design with four replications to study the effect of different levels of farmyard manure (FYM) and nitrogen on the yield and nitrogen uptake of *Stevia rebaudiana* Bertoni. Four levels of farmyard manure (0, 15, 30 and 45 t/ha) and four levels of nitrogen (0, 20, 40 and 60 kg/ha) were tested. Biomass yield (kg leaves/ha) and several other yield parameters (dry leaf yield, number of leaves per plant, leaf area index and dry matter accumulation per plant) were highest in plants grown at the highest level of farmyard manure (45 t FYM ha^{-1}). Maximum nitrogen uptake was also recorded under 45 t FYM ha^{-1} in both experiments. Plants grown at 40 and 60 kg N ha^{-1} produced significantly higher number of branches, number of leaves per plant, and showed higher leaf area index and nitrogen uptake compared to lower nitrogen levels. Dry leaf yield and dry matter accumulation per plant was highest at 60 kg N ha^{-1}.

Key words: Stevia, farmyard manure, nitrogen, yield, nitrogen uptake.

INTRODUCTION

Sugar forms an indispensable ingredient in the food habits of human being. The main source of sugar has for long been cane sugar with beet sugar contributing a small percentage. In India, the production of cane sugar is of the order of 240 million tonnes whereas that of beet sugar is 19500 tones. Though these sugars have sweetening qualities, they have been found to contribute to calories and are not advised for the consumption by diabetic patients. For these people, the world of sweetness has seen a sweeter change in the recent past with the introduction of stevia sugar obtained from leaves of stevia containing compounds about 250 to 300 times sweeter than the table sugar (Kumar, 2002). The chemicals of interest are stevioside, rebaudioside - A and at least six other compounds that have glucoside groups attached to a three carbon ring central structure. Stevioside concentration usually ranges from 3 to 10% of the leaf dry weight, whereas rebaudioside - A is less concentrated, ranging from 1 to 3%. Stevioside could be equivalent to the sweetening power of 28 tonnes per acre of sucrose sugar (Shock, 1982).

Stevia rebaudiana Bertoni, also known as the "sweetest plant of the world", belongs to the family Asteracea and is native to South American centre of diversity. Many countries have shown interest in cultivation of stevia and research activities have been initiated. Incorporation of

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this species into agricultural production systems however, depends upon a thorough knowledge of the plant and its agronomic potential (Ramesh et al., 2007). Nutritional requirements of this crop are low to moderate (Goenadi, 1985) since this crop is adapted to poor quality soils in its natural habitat at Paraguay. When placed under commercial culture, for economic purpose, manuring is necessary (Donalisio et al., 1982). Since plant leaves are the profitable part of this crop it is expected that a higher nutrient supply will result in higher foliage yield. However, the nutritional physiology of stevia is still poorly investigated, with only a few published studies. Here we investigate the effects of farmyard manure (FYM) and nitrogen level on the yield and nitrogen uptake of stevia. The results of this study provide additional information on the species nutritional requirements in order to obtain a better foliage yield.

MATERIALS AND METHODS

Two independent field experiments were conducted at the Student's Research farm of the Department of Agronomy, Punjab Agricultural University, Ludhiana, India one in 2006 and the other in 2007. The research farm is located 30°50'N, 75°52'E at an altitude of 247 m above mean sea level. The farm soil was loamy sand in texture, with organic carbon content and pH of 0.25/0.31% and 7.89/7.30, respectively in the years 2006/2007. The available nitrogen in the soil was 187 and 261 kg/ha, phosphorus was 15.3 and 18.6 kg/ha and potassium was 154 and 167 kg/ha in 2006 and 2007, respectively. The texture of the soil was analysed by International Pipette method (Piper 1966), organic carbon was analysed by Walkley and Black method (Piper, 1966), Available phosphorus was analysed by 0.5 M $NaHCO_3$ extractable (Olsen et al., 1954) and available potassium was analysed by ammonium acetate extractable K (Jackson, 1967). New experimental fields were designated for the 2007 experiment to avoid any residual effect of the farmyard manure applied in the previous year. The experiment was laid out in a completely randomized block design with four replications, in a total of 16 treatments consisting of four levels of farmyard manure (0, 15, 30 and 45 kg/ha) and four levels of nitrogen (0, 20, 40 and 60 kg/ha) and their interaction. The nutrient content of farmyard manure in the 2006 experiment was 0.49% N, 0.20% P_2O_5 and 0.38% K_2O, whereas in the 2007 experiment it was 0.51% N, 0.23% P_2O_5 and 0.40% K_2O. The required amount of farmyard manure as per treatment was weighed, added and well mixed in the soil a few days prior to the transplant of the seedlings. A basal dose of Phosphorus and potassium (40 kg/ha each) were added through single super phosphate and muriate of potash at the time of transplanting while nitrogen was applied as per treatment through urea. Nitrogen was applied in two equal splits, that is, half at the time of transplanting and remaining one-half was top dressed at 45 days after transplanting. On January 2006 and 2007, seeds of *S. rebaudiana* were sown on a seed bed by rubbing seeds with vermi-compost and covering them with farmyard manure and soil as the seeds have a very light weight. In April 2006 and May 2007, three-month old seedlings were then transplanted to the experimental plots, where they were grown under the experimental levels of farmyard manure and nitrogen. After transplanting, a light irrigation was given until the seedlings were well established, after which full irrigation was given to fulfill the water requirement of the crop. Any gaps between the plant and the soil were filled in order to maintain a straight plant position. During the experiment, the plants were protected from insects and checked regularly for any potential diseases. Choloropyriphos 20 EC at 5 L/ha was supplied by irrigation to control the termites and indofil at 0.3% (30 g in 10 L of water) was sprayed to control the leaf spot disease caused by *S. steviae*. Hand-weeding was done at 45, 75 and 95 days after transplanting. The crop was harvested manually in September 2006 and 2007 with the help of sickles. Plants were cut 15 cm above the ground level. Growth and yield parameters (number of branches per plant, number of leaves per plant, leaf area index) were recorded every month until harvest, whereas data on crop yield (biomass of dry leaves) and dry matter accumulation per plant were recorded at harvest, when the experiment ended. These parameters were chosen as important yield parameters of stevia because the leaves are the economic part of the plant. The plants harvested from each treatment were dried at 60°C till constant weight, ground, powdered and analyzed for nitrogen content using the alkaline permanganate method, according to Subiah and Asija (1956). Nitrogen uptake was estimated by multiplying yield and nitrogen content. The results are presented for each individual experiment (2006 and 2007). The data were statistically analyzed with the procedure described by Cochran and Cox (1967) and adapted by Cheema and Singh (1991) in statistical package CPCS-1 for significant differences between treatments.

RESULTS AND DISCUSSION

Number of branches per plant

Crop performance to a great extent is governed by the number of branches per plant. It is, therefore imperative that if the number of branches per plant is higher, the numbers of leaves are expected to be higher; ultimately the leaf yield will be higher. The number of branches per plant was significantly influenced by the different levels of farmyard manure and nitrogen. In the 2006 experiment, the application of 15 t FYM ha^{-1} resulted in a significantly higher number of branches $plant^{-1}$ (18.6) compared to control (16.7), whereas in 2007 significant increase in the number of branches $plant^{-1}$ were noticed at 30 t FYM ha^{-1}. Nitrogen fertilization influenced the number of branches per plant significantly during both the years. There was an increase in the number of branches per plant with each level increase in nitrogen. The highest number of branches per plant (18.9 and 19.0 during 2006 and 2007, respectively) was recorded under 60 Kg N ha^{-1} which was statistically at par with 40 and 20 Kg N ha^{-1} (18.8, 18.7 and 18.3, 17.9, respectively) and all the three levels of nitrogen produced significantly higher number of branches than control (16.8 and 16.3, respectively) during both years.

Number of leaves per plant

Green leaves are the site of photosynthetic activity taking place in the plants. The number of leaves per plant would also substantiate the fact that increased number of leaves per plant would contribute to the final yield of the plant particularly the crops like stevia in which only leaves are used for commercial product. The number of leaves per plant was significantly influenced by the different levels of farmyard manure and nitrogen. During 2006, the

Table 1. Influence of farmyard manure and nitrogen levels on yield attributes of *Stevia rebaudiana.*

Treatment	No. of branches/plant		Leaf no./plant		leaf area index (cm^2/cm^2)		Dry matter/plant(g/plant)	
	2006	2007	2006	2007	2006	2007	2006	2007
FYM (t ha^{-1})								
0	16.7	15.9	286.0	249.3	7.4	6.7	63.2	59.2
15	18.6	16.9	339.7	360.2	7.6	6.8	69.7	66.0
30	18.5	18.5	451.2	439.1	7.7	8.1	78.2	74.9
45	18.7	18.9	588.3	494.1	8.2	8.4	83.6	80.2
CD (P=0.05)	1.5	0.9	27.5	28.3	NS	0.4	9.8	9.7
Nitrogen (kg ha^{-1})								
0	16.8	16.3	354.1	359.1	7.7	6.9	47.2	43.2
20	18.3	17.9	394.1	367.8	7.7	7.3	65.4	61.3
40	18.8	18.7	432.9	386.7	7.8	7.8	81.9	78.4
60	18.9	19.0	484.0	429.0	7.8	8.0	97.3	100.3
CD (P=0.05)	1.2	1.4	27.5	28.3	NS	0.4	9.8	9.7
FYM × N	NS	NS	NS	NS	NS	NS	NS	NS

*CD, Critical difference; NS, non-significant.

maximum number of leaves per plant (588.0) was recorded with 45 t FYM/ha which was significantly higher than all other lower levels of farmyard manure, whereas, during 2007 the highest number of leaves count (494.1) was registered under 45 t FYM/ha which was statistically at par with 30 t FYM/ha but significantly higher than other lower levels (Table 1). The trend of increasing the number of leaves per plant with the application of farmyard manure was also recorded by Goenadi (1985). The application of increasing levels of farmyard manure increased the yield, which might have been due to the balanced availability of nutrients to the plants that resulted in a favorable soil environment. These favorable conditions increased the nutrient availability and the water holding capacity of the soil resulting in enhanced growth and yield.

The number of leaves per plant was significantly higher in plants grown at 40 and 60 kg N ha^{-1}, than at 0 and 20 kg N ha^{-1}. The maximum number of leaves per plant (484.0 and 429.0) was recorded with 60 kg N/ha which was significantly higher than other lower levels of nitrogen during both the years. The leaves count obtained under 60 kg N/ha were 302.3, 248.6, 137.1 and 244.8, 133.9 and 55 leaves per plant less than control, 20 and 40 kg N/ha during 2006 and 2007, respectively. Buana and Goenadi (1985) also reported increased number of leaves per plant with increased levels of nitrogen.

Leaf area index (cm^2/cm^2)

Leaf area index (LAI) is an important growth indices determining the capacity of plant to trap solar energy for photosynthesis and has marked influence on growth and yield of plant. The effect on leaf area index remained non-significant under different farmyard manure levels at all the growth stages during 2006. However, during 2007 the highest leaf area index (8.4) was obtained under 45 t farmyard manure/ha which was statistically at par with 30 t farmyard manure/ha (8.1) but significantly higher than 15 t farmyard manure/ha (6.8) and control (6.7) In case of nitrogen the maximum leaf area index (7.8) was recorded at 60 and 40 kg N/ha during 2006. In 2007, the maximum leaf area index (8.0) was obtained at 60 kg N/ha which was statistically at par (7.8) with 40 kg N/ha but significantly higher than 20 kg N/ha (7.3) and control (6.9).

Dry matter accumulation per plant (g/plant)

Dry matter accumulation by the crop is one of the important growth parameter of the crop to be considered for determination of the economic yield while assessing the effect of different treatments. Dry matter accumulation per plant significantly increased with the increment of farmyard manure in the two experiments. No significant differences were detected in dry matter accumulation between 30 and 45 t FYM ha^{-1}, the levels at which values were maximal for both years. These results corroborate the findings of Goenadi (1985). Similarly different levels of nitrogen significantly affected dry matter accumulation per plant during both the years. Maximum dry matter per plant (100.3 and 97.3 g/plant) was recorded at 60 kg N/ha which was significantly better than 40 and 20 kg N/ha and the lowest (47.2 and 43.1 g/plant) was obtained under control. These results are in close conformity with

Table 2. Influence of farmyard manure and nitrogen levels on yield and N uptake of *S. rebaudiana.*

Treatment	Dry leaf yield (kg/ha)		N uptake (kg/ha)	
	2006	2007	2006	2007
FYM t/ha				
0	802	596	16.98	14.58
15	846	680	21.33	17.00
30	909	793	25.71	22.15
45	1155	927	32.89	29.57
CD(P=0.05)	159.0	118.1	4.40	3.64
N kg/ha				
0	797	606	18.33	15.18
20	914	677	24.05	18.45
40	919	828	25.46	24.09
60	1080	885	29.06	25.59
CD(P=0.05)	159.0	118.1	4.40	3.6

the results obtained by Rakesh et al. (2012). Higher levels of nitrogen in the soil contributed to higher dry matter accumulation, which is an essential pre-requisite for photosynthetic ability in a given canopy and in turn might have helped other synthetic process during developmental sequence.

Dry leaf yield

No significant differences were detected in the dry leaf yield of plants grown at 30 and 45 t FYM ha^{-1}, when values were highest (respectively 909 and 1155 kg ha^{-1} in 2006 and 793 and 927 kg ha^{-1} in 2007) (Table 2). Plants grown at 60 kg N ha^{-1} produced the highest dry leaf yield (1080 kg ha^{-1}), which was significantly higher compared to all other levels of nitrogen. The increase in the dry leaf yield at 60 kg N ha^{-1} was 35.5, 18.1 and 17.5% higher over control, 20 and 40 kg N ha^{-1}, respectively, in 2006. The highest dry leaf yield, which was recorded with 60 kg N ha^{-1} (885 kg ha^{-1}) was not significantly different from that recorded at 40 kg N ha^{-1} (828 kg ha^{-1}) during 2007. Yet, the dry leaf yield obtained under these treatments was significantly higher than at 20 kg N ha^{-1} and in the control. Similar increase in dry leaf yield of stevia with nitrogen was also reported by Chalapathi et al. (1997).

The application of increasing levels of farmyard manure increased the yield, which might have been due to the balanced availability of nutrients to the plants that resulted in a favorable soil environment. These favorable conditions increased the nutrient availability and the water holding capacity of the soil resulting in enhanced growth and yield.

Maheshwar (2005) reported that application of 105:30:45 kg NPK/ha recorded significantly higher dry leaf yield due to maximum number of leaves per plant and branches per plant as compared to lower doses of nitrogen under loamy soil in Karnataka, India. Similarly, Lima Filho et al. (1997) observed that shortly before or at flowering, production of 1 t of dry leaves of *S. rebaudiana* required 64.6 kg N/ha, 7.6 kg P/ha and 56.1 kg K/ha. Lee et al. (1980) reported increase in leaf yield with moderate application of N, P and K fertilizers in Korea. There are, however, reports that stevia crop show yield reduction at high rates of fertilizer.

Nitrogen uptake

The uptake of nitrogen by stevia increased with increasing supply of farmyard manure. Maximum uptake was recorded under 45 t farmyard manure ha^{-1}, whereas uptake was lowest in plots where no farmyard manure was supplied. The higher uptake of nitrogen at 45 t farmyard manure ha^{-1} was attributed to a slow and prolonged availability of nutrients to the crop. In the nitrogen fertilization treatments, the highest N uptake was recorded when 60 kg N ha^{-1} was added, and was not statistically different from that obtained at 40 kg N ha^{-1}. However, the N uptake under these two treatments was significantly higher than that obtained at 20 kg N ha^{-1} and in the control for both years. The higher N uptake obtained at 40 and 60 kg N ha^{-1} may be related to the higher biomass yield obtained at the same N levels. The uptake of nitrogen increased probably because it was being used for plant growth. Angkapradipta et al. (1986) also reported that increased supply of nitrogen resulted in increased plant N content and nitrogen uptake by stevia.

Conclusion

From the results of present investigation, on the basis of dry leaf yield and total glycoside yield it may be concluded that the stevia crop may be supplied with 45 t farmyard manure/ha and 60 kg N/ha.

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