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Integrated effects of poultry manure and chemical fertilizer on the growth, leaf yield and stevioside content of stevia

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Abstract

Stevia is important for its leaves which are used as non calorie sweetener and also in many therapeutic applications including diabetes. The present investigation was carried out in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh to investigate the integrated effects of poultry manure (PM) and chemical fertilizer (CF) on the growth, leaf biomass yield and stevioside content of stevia grown in two contrasting soils (acid and non-calcareous) of Bangladesh. Six combinations of PM and CF viz. PM_0+CF_0, PM_0+CF_{100}, PM_3+CF_{50}, PM_3+CF_{75}, PM_5+CF_{50}, PM_5+CF_{75} were used. The study revealed that PM along with different percentages of chemical fertilizers exerted significant influence on the growth, leaf yield and stevioside content of stevia. The highest values of all the parameters except stevioside were found from the plant receiving PM @ 3 t ha^{-1} along with 75% CF. The leaf biomass yield was 1088% and 867% higher in acid soil and non-calcareous soil, respectively, over control. Stevioside content was increased with increasing the levels of PM up to 5 t ha^{-1} and also with the advancement of growth period from 30 to 60 days after planting. The highest amount of stevioside (10.45%) was detected in the leaves of the plant that received PM @ 5 t ha^{-1} along with 75% CF at 60 DAP in acid soil and it was 9.11% in non-calcareous soil at the same period. The lowest values of all the parameters including stevioside content were obtained from the plant which received neither PM nor CF. Considering the overall performance, farmers may be advised to cultivate stevia in acid soil applying PM @ 3 t ha^{-1} along with 75% chemical fertilizer to boost up the production of stevia under the agro-climatic condition of the study area in the context of Bangladesh.

Keywords: Stevia, Poultry manure, Chemical fertilizer, Leaf yield, Stevioside content, Acid and non-calcareous soil

Introduction

Stevia has been introduced as a crop in a number of countries and has become a popular natural source of high potency sweetener and dietary supplement. Cultivation of stevia crop made significant impact on the economy of many countries including Brazil, Korea, Mexico, United States, Indonesia, Tanzania and Canada (Lee *et al*., 1979; Donalisio *et al*., 1982; Goeinadi, 1983; Shock, 1982; Brandle and Rosa, 1992). Currently stevia production is concentrated in China and the major market is Japan (Kinghorn and Soejarto, 1985). Dry leaves are the economic part of stevia plant. The leaves synthesize zero-calorie ent-kaurene diterpene glycosides (steviosides and rebuadiosides). The major sweet compound stevioside as a dietary supplement for human subjects are manifold. It might be used as an alternative source to the synthetic sweetening agents like saccharine, aspertame, asulfam-K that are available in the market to the diet conscious consumers and diabetics (Aladakatti *et al*, 2012).

Stevia being a calorie free herb offers a solution for complex diabetic problems and obesity. The worldwide demand for the natural sweeteners is expected to increase in the years to come. Kinghorn and Soejarto (1985) opined that stevioside is a white amorphous powder present in leaf and stem tissue which was seriously considered as a cane sugar substitute. Literature survey revealed active constituents especially stevioside content in stevia plant greatly depends on the package of practices for the cultivation of stevia and adoption of modern agro- techniques and water management (Nepovim *et al*., 1998). Stevioside content varies from 4-20% depending on cultivar, soil, organic and inorganic fertilization and other environmental factors (Geuns, 2004). In view of the above fact, cultivation of stevia is gradually coming into focus in Bangladesh agriculture due to having no optimum agronomic management practices (Hasan, 2008 and Khan, 2014).

Some preliminary experiments on morphological and physiological parameters have been conducted in Bangladesh Sugarcrop Research Institute (BSRI). Recently *brac* has started pot and/or field experiment in small scale. Though *brac*, BSRI and Proshika have developed method for in vitro production of stevia seedlings, no study has yet been conducted to quantify the content of stevioside from stevia leaves in Bangladesh. Long term sustainable production of crops cannot be maintained by using chemical fertilizers alone and similarly, it is not possible to obtain higher crop yield by using only organic manure (Bair, 2000). Nambiar (2000) viewed that integrated use of manure and fertilizers would be quite promising not only in providing greater stability in production, but also in maintaining higher soil fertility status.

The problem of nutrient deficiencies as well as nutrient mining caused by intensive cropping and nutrient imbalance can be minimized by judicious application of chemical fertilizers in combination with organic manures. Poultry industry in Bangladesh has been increasing dramatically over last few decades and poultry manure (PM) has long been recognized as perhaps the most desirable animal manures because of its high nutrient content and ease of decomposition. Addition of PM to the crop field could be a very good option considering its availability in the context of Bangladesh for maintaining soil fertility and productivity. It is essential to develop an appropriate technique of cultivation and quantify the stevioside content to boost up the production of stevia in the country. The present research was, therefore, considered to investigate the integrated effects of poultry manure and chemical fertilizers on the growth, leaf biomass yield and stevioside content of stevia.

Materials and Methods

A pot experiment was conducted at the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh during March to May, 2011 to investigate the integrated effects of poultry manure (PM) and chemical fertilizer (CF) on the growth, leaf biomass yield and stevioside content of stevia. Two contrasting soils (acid and non-calcareous) were used. The physical and chemical properties of the soils can be seen from Zaman *et al*. (2015). Approximately 40 kg soils from each location (*Madhupur* for acid soil and *BAU farm* for non-calcareous soil) were collected from 0-15cm depth of selected fallow land for the experiment. The samples were made free from plant residues and other extraneous materials, air dried, ground and sieved through a 2mm sieve. Five hundred gram (500g) sieved soil from each source was preserved in a polythene bag and the physical and chemical properties were analyzed following standard procedure (Page *et al.* 1982).

Eight kg processed soil was taken in each earthen pot of 23 cm in height with 30 cm diameter at top and 18 cm at bottom leaving 3 cm from the top. *In vitro* produced 45 day old stevia seedlings (S*tevia rebaudiana* Bertoni) were collected from *brac* Biotechnology Laboratory, Joydebpur, Gazipur and used for the experiment. One stevia seedling was planted in each pot during 1st week of March, 2011. Nitrogen, P, K, S, Zn and B were applied as basal doses @ 250, 100, 200, 30, 3 and 1 kg ha^{-1} from TSP, MoP, gypsum, zinc sulphate and boric acid, respectively as chemical fertilizer (CF). Sulphur rate was adjusted from both gypsum and zinc sulphate. Following treatment combinations of PM and CF were used for the study.

Treatment symbol	Rate of PM (t ha^{-1}) and CF (%)
PM_0+CF_0	PM 0 plus CF 0
PM_0+CF_{100}	PM 0 plus CF 100
PM_3+CF_{50}	PM 3 plus CF 50
PM_3+CF_{75}	PM 3 plus CF 75
PM_5+CF_{50}	PM 5 plus CF 50
PM_5+CF_{75}	PM 5 plus CF 75

The experiment was laid out in completely randomized design with three replications. Total number of pots was 36 (6 treatment X 2 soil X 3 replication). Intercultural operations like irrigation, soil loosening, weeding, insect pest control, removal of flowers etc. were done as and when necessary. Data were collected at 15, 30, 45 and 60 days after planting (DAP). The crop was destructively harvested at 60 DAP. After harvesting the crop, leaf samples were separated, cleaned and dried at $60^{O}C$ for 72 hours, weighed, ground and stored. Plant height, branches plant^{-1}, leaves plant^{-1}, leaf area plant^{-1}, dry leaf weight of stevia leaves were studied. Stevioside content of stevia leaf was determined following the method of Kolb *et. al*.(2001). The results obtained were subjected to statistical analysis using standard method (Steel *et al*., 1997). The differences among the treatment means were compared by using Duncan Multiple Range test (Gomez and Gomez, 1984).

Results

The integrated effects of PM and CF on the growth, leaf biomass yield and stevioside content of stevia are described under the following heads.

Plant height

The application of PM and CF in different combinations had a significant effect on the plant height of stevia at different DAP in both the studied soils (Table 1). Plant height was gradually increased with time of growth period.

Table 1. Integrated effects of poultry manure and chemical fertilizer on the plant height of stevia at various DAP*

PM and CF level	Plant height (cm)									
	Acid soil					Non-calcareous soil				
	0	15	30	45	60	0	15	30	45	60
PM_0CF_0	9.0b	15.0c	27.3b	43.3c	55.3c	9.0b	16.3c	36.3b	58.3b	64.0c
$CF_{100}PM_0$	10.0ab	18.0b	40.0a	62.0a	77.7a	10.0ab	21.0ab	38.7b	62.0b	81.7b
PM_3+CF_{50}	10.0ab	15.0c	34.0ab	56.3b	75.0ab	10.0ab	20.0b	37.3b	61.7b	78.3bc
PM_3+CF_{75}	10.0ab	20.3a	40.7a	65.7a	83.0a	10.3a	24.0a	50.7a	78.0a	94.7a
PM_5+CF_{50}	9.0b	17.3b	37.7a	61.3a	72.7b	9.0b	22.7ab	48.0a	73.0a	76.7bc
PM_5+CF_{75}	10.3a	20.0a	36.0ab	59.3ab	69.7bc	10.3a	22.0ab	39.3b	62.0b	73.3bc
CV(%)	0.9	1.2	1.2	1.2	1.2	0.9	1.1	1.3	1.1	1.2
$LSD_{0.05}$	1.1	1.9	3.9	6.1	7.6	1.2	2.1	4.5	6.8	8.1

*DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference,

The tallest plant (83cm in acid soil and 94.7cm in non-calcareous soil) was recorded from the treatment PM_3CF_{75} at 60 DAP in both soils under study which was identical with all other combinations of PM and CF except control in acid soil at the same DAP but significantly different from other treatment combinations of PM and CF in non-calcareous soil. The shortest plant (55.3cm in acid soil and 64cm in non-calcareous soil) was noticed in control treatment (PM_0CF_0).

Branch number

Significant variation on the number of branches plant^{-1} was observed due to the application of different combinations of PM and CF at various DAP (Table 2). Branch numbers increased slowly up to 15 DAP and then increased rapidly up to 60 DAP.

The highest number of branches plant^{-1} (13 and 15.3 in acid and non-calcareous soil, respectively) was observed in the plant fertilized with PM @ 3 t ha^{-1} in combination with 75% CF where as the lowest branch number was observed throughout the growth period in the control treatment. The second highest number of branches (11 in acid soil and 10.3 in non-calcareous soil) was counted from PM_5CF_{50}.

Table 2. Integrated effects of poultry manure and chemical fertilizer on the branch number of stevia at various DAP*

PM and CF level	Branches $Plant^{-1}$ (No.)									
	Acid soil					Non-calcareous soil				
	0	15	30	45	60	0	15	30	45	60
PM_0CF_0	1.0	1.3b	1.7c	2.0c	2.7c	1.0	1.3c	2.0c	2.3c	3.0c
$CF_{100}PM_0$	1.0	2.0b	5.0a	7.3a	9.0b	1.0	2.0b	5.0ab	7.0b	9.7b
PM_3+CF_{50}	1.0	1.7b	4.7ab	6.0ab	7.7b	1.0	1.7bc	4.3b	6.0b	8.0bc
PM_3+CF_{75}	1.0	3.0a	6.0a	8.0a	13.0a	1.0	4.0a	7.0a	10.0a	15.3a
PM_5+CF_{50}	1.0	2.3a	3.3b	6.0ab	11.0ab	1.0	3.3ab	6.3a	8.0a	10.3b
PM_5+CF_{75}	1.0	2.0b	3.0bc	5.0b	9.0b	1.0	2.0b	5.0ab	7.7ab	9.0b
CV(%)	0.0	3.3	3.6	3.6	3.4	0.0	3.9	3.6	3.4	3.6
$LSD_{0.05}$	0.0	0.8	1.2	2.2	2.1	0.0	0.8	2.0	2.1	2.3

*DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference

Leaf number

Leaf number differed significantly due to the application of different levels of PM and CF at various DAP (Table 3). A rapid and tremendous increase in leaf number was observed in the plant fertilized with PM @ 3 t ha^{-1} along with 75% CF in both soils. The highest number of leaves $plant^{-1}$ was counted from the plant treated with PM_3CF_{75} which was significantly different from other treatment combinations. In contrast, the lowest leaf number was observed in the plant receiving neither PM nor CF. Plants fertilized with PM_0CF_{100}, PM_3CF_{50}, PM_5CF_{50}, PM_5CF_{75} produced identical number of leaves $plant^{-1}$ in non-calcareous soil. Except control, among other treatment combinations, the lowest number of leaves was counted from the plant fertilized with PM_5CF_{75} which was significantly different from other treatments in acid soil.

Table 3. Integrated effects of poultry manure and chemical fertilizer on the leaf number of stevia at various DAP*

PM and CF level	Leaves $Plant^{-1}$ (No.)									
	Acid soil					Non-calcareous soil				
	0	15	30	45	60	0	15	30	45	60
PM_0CF_0	4.3	8.0e	22.0d	25.7d	27.7d	4.3	11.3e	26.3c	38.7d	46.3d
$CF_{100}PM_0$	5.0	26.0b	78.0b	189.0b	309.0ab	5.0	31.7b	89.3b	213.0b	359.0b
PM_3+CF_{50}	4.7	20.0c	60.3c	156.0c	280.0b	4.7	25.3c	82.3b	199.0bc	320.0bc
PM_3+CF_{75}	4.7	35.0a	91.0a	256.0a	330.0a	5.0	39.3a	121.7a	309.0a	448.0a
PM_5+CF_{50}	4.7	17.7c	61.3c	165.7bc	288.0b	4.7	24.3c	97.0b	191.0c	346.0b
PM_5+CF_{75}	5.0	11.7d	55.0c	157.3c	207.0c	4.3	15.0d	86.0b	178.0c	298.0c
CV(%)	2.3	3.8	2.9	3.6	3.5	2.3	3.2	2.9	3.5	3.4
$LSD_{0.05}$	1.6	2.6	6.6	17.1	26.4	1.6	3.0	8.8	20.4	32.0

*DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference

Leaf number was increased by 179-302 in acid soil and 252-402 in non-calcareous soil over control irrespective of treatments.

Leaf area

The application of PM and CF in different combinations had a significant effect on the leaf area of stevia at 60 DAP (Table 4). The highest leaf area $plant^{-1}$ (2307 cm^2 in acid soil and 4115 cm^2 in non-calcareous soil) was measured from the plant fertilized with PM_3CF_{75} in both soils which was identical with all other combinations of PM and CF except control in non-calcareous soil at the same DAP but significantly different from PM_5CF_{75} and control in acid soil. The lowest leaf area $plant^{-1}$ (133 cm^2 in acid soil and 246 cm^2 in non-calcareous soil) was noticed in the plant grown in control treatment (PM_0CF_0).

Dry weight

The dry weight of stevia leaves plant^{-1} at harvest varied significantly due the application of different levels of PM and CF (Table 4). Results revealed that dry weight progressively increased with increasing levels of PM and CF application up to PM_3CF_{75} in both soils and then declined with further addition (PM_5CF_{50} and PM_5CF_{75}). The highest dry weight plant^{-1} (9.98g in acid soil and 13.55g in non-calcareous soil) at harvest was measured from the plant receiving PM_3CF_{75} which was significantly higher than other levels of PM and CF in non-calcareous soil but identical with PM_0CF_{100} and PM_5CF_{50} in acid soil.

Table 4. Integrated effects of poultry manure and chemical fertilizer on leaf area, dry weight and yield increase of stevia leaves over control at harvest

PM and CF level	Leaf area plant^{-1} (cm^2)		Leaf dry weight (g plant^{-1})		Yield increase over control (%)	
	Acid soil	Non-calcareous soil	Acid soil	Non-calcareous soil	Acid soil	Non-calcareous soil
PM_0CF_0	133c	246c	0.84d	1.40d	-	-
PM_0CF_{100}	2076ab	2471b	9.35a	10.86b	1013	675
PM_3+CF_{50}	1589b	2267b	8.47b	9.68c	908	591
PM_3+CF_{75}	2307a	4115a	9.98a	13.55a	1088	867
PM_5+CF_{50}	1661b	2399b	8.71b	10.47b	937	648
PM_5+CF_{75}	1352b	2558b	6.26c	9.01c	645	543
CV(%)	4	4	3.52	3.38	-	-
$LSD_{0.05}$	445	460	0.81	1.01	-	-

CV = Coefficient of variance, LSD = Least significant difference

Second highest values (9.35g in acid soil and 10.86g in non-calcareous soil) were obtained from PM_0CF_{100}. The leaf yield was 41% higher in respect of combined application of PM @ 3 t ha^{-1} and CF 75% than that of the sole application of PM maintaining the equal rate. The lowest values were obtained from the control treatment (PM_0CF_0). Dry weight increased by 645 to 1088% in acid soil and 543 to 867% in non-calcareous soil over control across the treatments.

Stevioside content

There was a significant effect of different levels of PM and CF on the stevioside contents of stevia leaf (Table 5). Stevioside content of the leaf was gradually increased with increasing the levels of PM irrespective of soils used.

Table 5. Integrated effects of poultry manure and chemical fertilizer on the stevioside contents of stevia leaf at various DAP

PM and CF level	Stevioside content (%)					
	Acid soil			Non-calcareous soil		
	30 DAP	45 DAP	60 DAP	30 DAP	45 DAP	60 DAP
PM_0CF_0	2.96d	3.72e	5.22e	2.49e	3.25e	5.10c
PM_0CF_{100}	3.28d	4.45d	5.98d	3.00d	3.97d	5.63c
PM_3+CF_{50}	4.62c	5.21c	7.37c	4.12c	5.00c	6.82b
PM_3+CF_{75}	4.93c	6.19b	8.63b	4.36c	5.72b	7.14b
PM_5+CF_{50}	5.75b	6.36b	9.14b	5.27b	6.04b	8.37a
PM_5+CF_{75}	6.24a	8.25a	10.45a	5.92a	7.82a	9.11a
CV(%)	2.19	2.19	1.99	2.39	2.36	1.75
$LSD_{0.05}$	0.48	0.60	0.82	0.45	0.55	0.74

DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference,

The highest stevioside content (10.45% in acid soil and 9.11% in non-calcareous soil) was found in the leaves of the plant fertilized with PM @ 5 t ha^{-1} along with 75% CF which was at par with the stevioside contents of the plants receiving PM @ 5 t ha^{-1} and 50% CF in both soils. The lowest stevioside content was obtained from the plant fertilized with neither PM nor CF. Stevioside contents were also significantly increased with the advancement of growth period from 30 to 60 DAP irrespective of treatments and soils used. Leaf stevioside content of the PM amended soil was much higher than the plants grown in CF amended soil. Plants grown in acid soil synthesized more stevioside compared to the plants of non-calcareous soil irrespective of the treatments employed.

Discussion

Leaf yield and yield attributes

The highest plant height was observed in PM_3+CF_{75} treatment at harvesting time followed by the treatment PM_0+CF_{100}, PM_3+CF_{50}, PM_5+CF_{50}, PM_5+CF_{75} and PM_0+CF_0 treatments, respectively. The reasons of obtaining higher plant height might be due to the combined application of chemical fertilizer and poultry manure. On the other hand, the lowest plant height of the control treatment might be due to the application of neither organic nor chemical fertilizers. Kumar *et al*. (2013) noticed that combined application of chemical fertilizer and poultry manure increased the plant height of stevia. The number of branches plant^{-1} was gradually increased under all treatments. The growth was much higher under non calcareous soil condition than acid soil (Zaman *et al*., 2015). The number of the branches was higher in PM_3+CF_{75} treatment for supplying sufficient nutrient throughout the growth period from poultry manure and chemical fertilizers. The number of leaves plant^{-1} increased slowly in earlier days due to establishment and after that the abrupt growth in number of leaves was noticed. The highest number of leaves plant^{-1} was found in PM_3+CF_{75} under non-calcareous soil condition throughout the growth period. However, it is clear that number of leaves plant^{-1} was higher in the treatment where organic manure and chemical fertilizers (CF) were applied as integrated pattern.

Different doses of organic and inorganic fertilizers significantly influenced the leaf parameters like leaf area. Leaf characteristics viz. leaf length, breadth and area as influenced by different levels of N, P, K and S fertilizers along with the organic manure like CD, PM and VC in acid and non-calcareous soils. Maheshwar (2005) and Khanom *et al.* (2008) observed enhanced leaf parameters with increased levels of fertilizers. Either sole application of CF or combined application of CF and organic manures like cow dung (CD), PM or vermicompost (VC) have the important roles in increasing the leaf fresh and dry weight of any crop. The biomass yield was maximum due to the application of different organic and inorganic fertilizers both in acid and non-calcareous soil. This is also identical with Angkapradipta *et al.* (1986) and Nasrin (2008), where the increased biomass production was achieved due to the application of higher levels of N fertilizers. Aladakatti (2012) reported increased biomass production using different levels of fertilizers. Fallah *et al*. (2006) carried out an experiment and concluded that use of VC significantly increased the fresh weight of stevia leaves.

Stevioside content

Results showed that the content of stevioside have been found to be increased significantly with the increase of biomass content up to 60 DAP irrespective of treatments. However, the results further envisaged that the percentage content of stevioside has been recorded highest in the combined application of PM and CF (10.45%) over that of corresponding sole application of CF and that of control treatment. It was observed that the stevioside content was increased with the increased rate of PM from 3 to 5 t ha^{-1} along with CF irrespective of growth period. The results are in accordance with the findings of Das and Dang (2010) who found increased amount of stevioside with advanced growth period and highest amount of stevioside in the stevia leaf of the bio-fertilizer treated plant.

The results indicate that the leaf biomass yield and stevioside content in stevia leaf has been markedly increased due to PM application either as single or in combination with CF. However, the overall results suggest that the combined application of PM and CF was always superior as compared to sole application of CF which enhanced significant foliage growth of stevia leaves. In other way, the HPLC study revealed the presence of stevioside in the stevia extract in acidic soil and interestingly, the magnitude of increased stevioside content was higher for stevia extracts collected from treatment PM_5CF_{75} where combination of both fertilizers were applied and that was due to increased amount of organic carbon and other macro and micronutrient contents in the acidic soil with PM (Das and Dang, 2010).

The stevioside was accumulated in the leaves throughout the growth period of the stevia and concentrated particularly at the later growth stage may be due to higher leaf area and high net photosynthetic rate. Every physiological indexes of the stevia in the PM amended plant were significantly higher than the CF application. That could be the main reason of higher stevioside content of the PM amendment over that of CF addition. Moreover, poultry manure can promote the over-ground growth, root vigour, leaf net photosynthetic capability and the total glycoside contents in leaf of the stevia (Liu and Shi, 2011).

Conclusion

The study revealed that PM along with different percentages of chemical fertilizer exerted significant influence on the growth, leaf yield and stevioside content of stevia. The highest values of all the parameters except stevioside content were found from the plant receiving PM @ 3 t ha^{-1} along with 75% chemical fertilizer. Stevioside content was increased with higher levels of PM up to 5 t ha^{-1} along with the advancement of growth period from 30 to 60 DAP. The highest amount of stevioside (10.45%) was found in the leaves of the plant that received PM @ 5 t ha^{-1} along with 75% CF. Considering all the studied parameters and treatments, it can be concluded that stevia could be cultivated in acid soil applying PM @ 3 t ha^{-1} along with 75% chemical fertilizer for producing higher leaf biomass yield of stevia.

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