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Effect of cow dung as organic manure on the growth, leaf biomass yield of *Stevia rebaudiana* and post harvest soil fertility

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Abstract

Organic manures as a source of plant nutrients for cultivation of field crops has received worldwide attention due to rising costs, rapid nutrient loss and adverse environmental impacts from inorganic fertilizers. A pot experiment was conducted in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh to observe the effects of cow dung on growth, yield of stevia along with post-harvest fertility status of soil. Four treatments of CD *viz.* 0, 5, 7.5 and 10 t ha^{-1} in two contrasting soils (acid and non-calcareous) were examined following Completely Randomized Design (CRD) with three replications. Growth and yield attributes increased significantly with the advancement of growth period (60 DAP) and increased rate of CD up to 10 t ha^{-1}. An overall performance of non-calcareous soil was better than acid soil. In non-calcareous soil, the leaf biomass yield was increased by 275% whereas in acid soil it was 268% over control. The acidity of both soils significantly decreased with the increased rate of CD. All essential plant nutrients *viz.* total N, available P exchangeable K, Ca, Mg, available S, Zn, B and organic matter content of soil were significantly increased with the increased levels of CD up to its highest dose (CD @ 10 t ha^{-1}) in both soils. Thus from the findings of the present research work it can be concluded that for getting optimum leaf biomass yield of stevia along with fertility of both soils CD should be applied @ 10 t ha^{-1}.

Introduction

Stevia, the nature's sweetest gift, has numerous health benefits (Rashid *et al.*, 2013). Stevia is approximately 20 to 30 times sweeter than common table sugar, calorie-free and non-disruptive to blood sugar levels without any side effects. It is also 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). Recently in many countries stevia becomes a popular source of dietary supplement. As Bangladesh is mainly an agro-based country, stevia cultivation has an immense scope for intensive agriculture and precision farming and fits well for high return agriculture (Barathi, 2003). Now it becomes a new avenue to do research for the researcher and is a good opportunity for the farmers to earn cash money through stevia. Incorporation of stevia into agricultural production systems depends upon details information regarding the plant, its agronomic potential and nutritional requirement (Ramesh *et al.*, 2007). Unfortunately no detailed study has yet been conducted on suitability of soil, organic and inorganic fertilizer requirement, pest management, chemical and/or biochemical constituents etc. for large scale cultivation of stevia in Bangladesh. Some preliminary experiments on morphological and physiological parameters have been conducted by BSRI and *brac*. Few sporadic trials on the growth and leaf yield of stevia have been conducted both at pot and field conditions (Hasan, 2008; Khan, 2014). Recently, suitable soil (Zaman *et al.*, 2015a), N and S requirement, critical N and S content (Zaman *et al.*, 2016a and 2016b) and P use efficiency and critical P content (Zaman *et al.*, 2017) in stevia grown in both acid and non-calcareous soil has been reported in Bangladesh.

Amendment of organic materials such as crop residues, animal manures, green manures to soils directly affect soil organic matter content, soil fertility, soil physical characteristics, and augmentation of microbial activities, amelioration of metal toxicity (Escobar and Hue, 2008; Roy and Kashem, 2014 and Wong and Swift, 2003). Cow dung has long been recognized as perhaps the most desirable animal manures because of its high nutrient and organic matter content. Addition of cow dung increases the organic carbon content of degraded soil which may lead to the increasing activity of beneficial soil microorganisms as well as the fertility status of soil by increasing the availability of nutrients for the plants from soil. Cow dung significantly increased the growth and yield of plants (Gudugi, 2013; Akande *et al.*, 2006; Mehedi *et al.*, 2011). This study therefore sought to evaluate the effects of cow dung on growth, leaf biomass yield of stevia and its effect on the fertility status of post harvest soil in the ecological condition of Bangladesh Agricultural University.

Materials and Methods

The experiment was carried out with stevia plant in the Department of Agricultural Chemistry, BAU, Mymensingh during February to May, 2012. The research work was accomplished in 24 earthen pots in a completely randomized design with three replications. Two soils (acid and non-calcareous) of contrasting physicochemical properties (Zaman *et al.*, 2015a) collected from Madhupur (Tangail) and BAU campus (Mymensingh), respectively were used. The soil was mixed thoroughly with cow dung (CD) as per treatments @ CD_0 (control), CD_5 (5 t ha^{-1}), $CD_{7.5}$ (7.5 t ha^{-1}) and CD_{10} (10 t ha^{-1}). The CD contained 24.03, 1.05, 0.35, 0.45, 0.24, 0.16 and 0.015% organic C, total N, P, K, S, Ca and Mg, respectively. Eight kg of processed soil was taken in each earthen pot. Forty five days old *in vitro* produced stevia seedlings were collected from BRAC Biotechnology Laboratory, Joydebpur, Gazipur to use in the experiment. For the initial growth and development of the seedling, small amounts of urea were applied to each pot including control. Intercultural operations like irrigation, soil loosening, weeding, plant protection and deflowering were done as and when necessary. Harvesting, cleaning, drying and weighing were done properly. Plant height, number of branches, number of leaves, leaf area, fresh leaf weight and dry leaf weight $plant^{-1}$ were studied at 15 days interval. Initial and post-harvest soil properties were determined following standard methods (Page *et al.*, 1982) in the Laboratories of the Departments of Agricultural Chemistry, Biochemistry, Professor Muhammed Hussain Central Laboratory (PMHCL), BAU, Mymensingh and SRDI Regional Laboratory, Dhaka. Analysis of variance (ANOVA) was done following the principle of F-statistics and the mean values were separated by Latin Square Design (LSD) (Gomez and Gomez, 1984).

Results and Discussion

Effect of CD on plant height

Amendments of organic matter in soil significantly affect plant height. Effects of different CD doses on plant height in both acid and non-calcareous soil is given in Table 1. The application of CD influenced plant height and it was increased with the advanced doses of CD. The shortest plants were recorded with control in both soils. However, the tallest plant (98cm in acid soil and 94cm in non-calcareous soil) was found in the pots receiving higher CD dose @ 10 t ha^{-1}. Gudugi (2013) reported similar result in okra and concluded that CD @ 20 t ha^{-1} was responsible for getting the tallest plant. Height increase was 58% higher in acid soil and 57% higher in non-calcareous soil over control. It was observed that the pots subjected to $CD_{7.5}$ and CD_{10} were significantly higher than other treatments at 60 DAP.

Table 1. Effects of different levels of cow dung (CD) on the plant height of stevia at various DAP

CD level	Acid soil					Non-calcareous soil				
	DAP					DAP				
	0	15	30	45	60	0	15	30	45	60
CD_0	10.0b	16.3b	33.0c	49.0b	62.0c	13.3	19.3c	43.0c	51.7b	60.0c
CD_5	11.7a	21.7ab	44.7b	61.3b	77.0b	14.0	21.0bc	48.0bc	58.3b	73.3bc
$CD_{7.5}$	12.7a	24.7a	53.0ab	77.7a	87.7ab	15.0	24.7ab	53.0ab	77.3a	84.3ab
CD_{10}	13.0a	26.7a	60.0a	80.3a	98.3a	15.0	27.0a	56.7a	82.0a	93.7a
CV(%)	2.1	2.0	2.3	2.1	1.8	1.3	1.6	1.3	2.0	1.8
$LSD_{0.05}$	2.8	3.2	4.9	7.4	8.1	2.3	2.6	4.8	6.6	8.3
SE±	0.7	1.4	3.3	4.2	4.5	0.6	1.1	1.9	4.1	4.2

DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means

Effect of CD on branch number

It is well established that the addition of organic fertilizers increased the organic matter contents of the soil and availability of other plant nutrients (Brar *et al.*, 2004). For this reason the number of branches $plant^{-1}$ was significantly increased due to the application of different levels of CD in both acid and non-calcareous soil (Table 2). The result revealed that branches $plant^{-1}$ progressively increased with increasing levels of CD up to 10 t ha^{-1} in both soils. The highest number of branches $plant^{-1}$ at 60 DAP was counted from the plant receiving 10 t CD ha^{-1} which was identical with $CD_{7.5}$ but significantly different from other treatments. The lowest branch number was counted from control.

Table 2. Effects of different levels of cow dung (CD) on the branch number of stevia at various DAP

CD level	Acid soil					Non-calcareous soil				
	DAP					DAP				
	0	15	30	45	60	0	15	30	45	60
CD_0	1.0	1.3b	1.7b	1.7b	1.7b	1.0	1.3b	1.3b	1.7c	1.7c
CD_5	1.0	2.0a	2.3a	2.3a	3.3a	1.0	2.3ab	2.7ab	2.7bc	3.0bc
$CD_{7.5}$	1.0	1.7a	2.7a	3.0a	3.7a	1.0	3.0a	3.7a	3.7ab	4.0ab
CD_{10}	1.0	2.3a	3.0a	3.3a	4.0a	1.0	3.3a	4.0a	4.3a	4.7a
CV(%)	0.0	3.8	3.6	3.4	3.4	0.0	3.9	4.3	3.9	4.0
$LSD_{0.05}$	0.0	0.8	0.9	0.8	0.8	0.0	0.8	0.9	0.8	0.9
SE±	0.0	0.2	0.3	0.3	0.3	0.0	0.3	0.4	0.4	0.4

DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means

The result was analogus to the findings reported by different researchers (Brar *et al.* 2004; Al- Mustafa *et al*., 1995 and Singh and Singh 1999). Ailincăi *et al.* (2010) also reported highest increase in the number of tillers of wheat and different horticultural crops treated with high dose of oilcake and sewage sludge.

Effect of CD on leaf number

The data pertaining to the number of leaves plant^{-1} as influenced by different levels of CD in both acid and non-calcareous soils at various DAP have been presented in Table 3. Application of CD at different rates showed significant effect on the number of leaves of stevia plants at all growth stages except 0 DAP irrespective of soils and treatments used. Leaf number increase was very slow at the early growth stages (0–30 DAP) while it was rapid between 30 and 60 DAP irrespective of CD levels except control.

Table 3. Effects of different levels of cow dung (CD) on the leaf number of stevia at various DAP

CD level	Acid soil					Non-calcareous soil				
	DAP					DAP				
	0	15	30	45	60	0	15	30	45	60
CD_0	6.3b	9.7d	16.0d	24.0d	45.0c	5.7b	12.3c	22.0c	31.3d	48.0d
CD_5	8.3a	19.0c	31.3c	57.3c	90.0b	8.3a	25.0b	39.7b	64.7c	95.0c
$CD_{7.5}$	9.3a	25.0b	38.0b	70.0b	100.0b	9.0a	36.7a	46.0b	99.3b	125.0b
CD_{10}	9.7a	33.0a	45.0a	88.0a	159.0a	9.7a	40.7a	60.0a	115.3a	170.0a
CV(%)	2.9	4.1	3.4	4.0	4.2	3.2	4.0	3.4	4.3	4.2
$LSD_{0.05}$	2.7	2.8	3.7	6.6	11.6	2.8	3.4	4.6	8.5	12.3
SE±	0.7	2.7	3.3	7.2	12.5	0.8	3.4	4.3	10.0	13.7

DAP = Days after planting, CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means

The main limiting factor for slower growth at early stage was the nutrient deficiency in the soil as the organic manure had not been fully decomposed. But there was a contrary situation in the later growth stage, the stevia of the organic manure cultivation got sufficient nutrient; so the growth was rapid and number of leaves plant^{-1} was higher. Maximum number of leaves (159.0 in acid soil and 170.0 in non-calcareous soil) at harvest was recorded with CD_{10} which was significantly higher than all other levels of CD in both soils. At harvest, second highest number of leaves (100.0 and 125.0 in acid and non-calcareous soils, respectively) was recorded with $CD_{7.5}$ which was identical with CD_5 in acid soil but significantly different with others in non-calcareous soil. The minimum number of leaves plant^{-1} was harvested from the plants fertilized with no CD irrespective of soils and growth period. Wang *et al.* (2013) also reported similar result i.e. number of leaves plant^{-1} was affected by various levels of CD though CD @ 15 t ha^{-1} produced maximum number of leaves. Mujahid and Gupta (2010) used oilcake to enhance the leaf structure, number and quality of lettuce. Akdeniz *et al*. (2006) reported that sewage sludge application 10 t ha^{-1} positively affected grain yield, leaf number, leaf nitrogen, harvest index, and total N uptake by sorghum more than other doses.

Effect of CD on leaf area

Leaf area plant^{-1} responded variably due to the application of different levels of CD (Table 4). There were steady increase in the leaf area of stevia was found with increasing CD doses. The highest total leaf area plant^{-1} (973 cm^2 in acid soil and 1511cm^2 in non-calcareous soil) at harvest was measured from the plant receiving CD @ 10 t ha^{-1}. Second highest values (551 cm^2 in acid soil and 969 cm^2 in non-calcareous soil) were obtained from $CD_{7.5}$. Identical leaf area was also obtained from the plants fertilized with $CD_{7.5}$ and CD_5 in non-calcareous soil. Similar type of result was found by Uka *et al*. (2013) in the cultivation of okra. The lowest leaf area was found from the control treatment irrespective of soils used. N application at all levels increased leaf area by 109-367% in acid soil and 154 to 488% in non-calcareous soil, respectively at harvest.

Table 4. Effects of different levels of cow dung (CD) on leaf area, dry weight and yield increase of stevia leaves over control at harvest

CD level	Leaf area plant^{-1} (cm^2)		Leaf dry weight (g plant^{-1})		Yield increase over control (%)	Yield increase over control (%)
	Acid soil	Non-calcareous soil	Acid soil	Non-calcareous soil	Acid soil	Non-calcareous soil
CD_0	208d	257c	1.35c	1.44d	-	-
CD_5	435c	654b	2.71b	2.85c	101	98
$CD_{7.5}$	551b	973b	3.05b	3.90b	126	171
CD_{10}	973a	1511a	4.97a	5.44a	268	278
CV(%)	4	5	4.39	4.40	-	-
$LSD_{0.05}$	66	197	0.35	0.40	-	-
SE±	69	119	0.40	0.45	-	-

CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means

Effect of CD on dry weight

Dry weight progressively increased with increasing levels of CD application up to 10 t ha^{-1} in both soils (Table 4). The highest (4.97g in acid soil and 5.44g in non-calcareous soil) and the second highest (3.05g in acid soil and 3.90g in non-calcareous soil) dry weight $plant^{-1}$ at harvest were measured from the plant receiving maximum level of CD_{10} and $CD_{7.5}$, respectively. In acid soil stevia receiving $CD_{7.5}$ and CD_5 doses showed identical result. The lowest values were recorded from the control treatment. CD application at all levels increased leaf dry yield at harvest by 101 to 268% in acid soil and 98 to 278% in non-calcareous soil, respectively over control. Tanimu *et al*. (2007) confirmed similar result in case of Maize. El-Dewiny *et al.* (2006) showed that dry weight of radish and spinach plants increased with application of sewage sludge.

Effect of CD on fresh weight

The fresh weight of stevia leaves $plant^{-1}$ at harvest varied significantly due the application of different levels of CD (Fig. 1). Results revealed that fresh weight progressively increased with increasing levels of CD. The highest fresh weight $plant^{-1}$ (17.72g in acid soil and 18.96g in non-calcareous soil) at harvest was measured from the plant receiving 10 t CD ha^{-1} which was significantly higher than other levels of CD. The lowest values were obtained from the control treatment (5.01g in acid soil and 5.35g in non-calcareous soil). CD application at all levels increased fresh weight at harvest by 5.11 to 12.71g $plant^{-1}$ in acid soil and 5.25to 13.61g $plant^{-1}$ in non-calcareous soil.

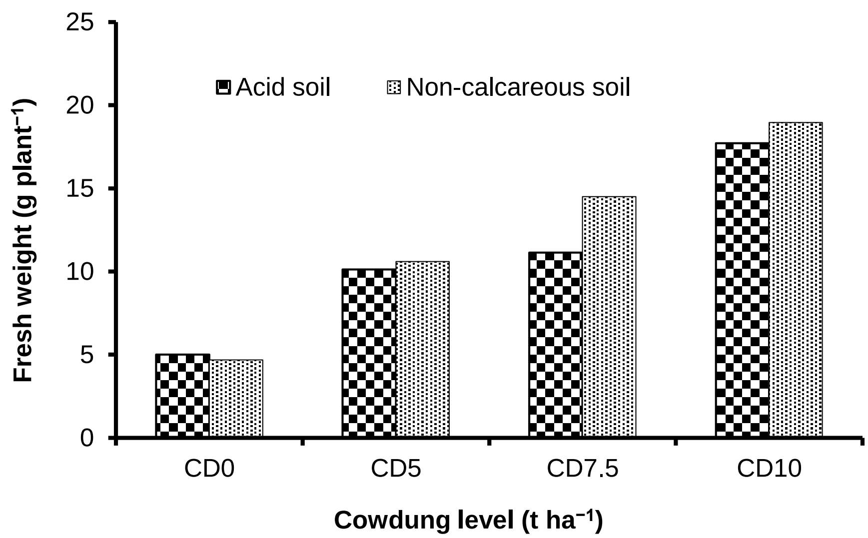

Fig. 1. Effects of different levels of cow dung (CD) on the fresh weight of stevia leaves at harvest

The finding of the present study was homologous as the findings reported by Zaman *et al*., (2015b) using vermicompost as organic additives. Mehedi *et al.* (2011) also described highest yield of carrot applying CD @ 15 t ha^{-1}. El-Dewiny *et al.* (2006) showed that fresh weight of radish and spinach plants increased with increased application of sewage sludge. The fresh weight of leaves varied with levels and types of organic amendment irrespective of soils used. Similar result was reported by Uka *et al*. (2013) for the fresh weight of okra.

Effect of CD on post harvest fertility of soil

The data pertaining to post harvest soil properties as influenced by different levels of CD application have been presented in Table 5a and 5b. It can be seen from the Tables that all the parameters were significantly influenced by the addition of CD irrespective of soils used. The values of the soil parameters as expected were increased with the increased levels of CD. The acidity of both soils was reduced to some extent and favors the growth and yield of stevia. At all CD levels, pH increased by 0.3 to 0.5 units in both soils. Wang *et al.* (2013) depicted the same opinion that any organic material if added to the soil that will reduce soil acidity. They reported that addition of the plant residues increased soil pH by 0.1–0.8 U. This might be due to the fact that when organic residues (plant or animal) are added to the soil, they release organic anions which neutralize the hydrogen ion of the acid soil.

Table 5a. Effects of different levels of cow dung (CD) on the fertility of post harvest soils

Levels of CD	pH		Organic matter (%)		Total N (μg g^{-1})		Available P (μg g^{-1})		Exch. K (Cmol kg^{-1})	
	AS	NS	AS	NS	AS	NS	AS	NS	AS	NS
CD_0	4.9b	6.5c	1.70b	1.28b	0.10b	0.12b	2.83c	10.00c	0.18b	0.15b
CD_5	5.2a	6.8b	1.95b	1.98a	0.15b	0.17b	3.90b	13.16c	0.21ab	0.18ab
$CD_{7.5}$	5.3a	7.0a	2.26ab	2.51a	0.18a	0.21a	4.96b	16.18b	0.25a	0.22a
CD_{10}	5.4a	7.2a	2.55a	2.96a	0.23a	0.24a	6.00a	19.19a	0.28a	0.25a
CV(%)	3.0	3.1	2.52	4.29	3.59	5.30	3.92	5.42	4.62	5.10
$LSD_{0.05}$	0.4	0.4	0.31	0.35	0.04	0.05	0.39	2.35	0.07	0.05
SE±	0.1	0.2	0.08	0.13	0.02	0.01	0.09	0.91	0.01	0.01

AS = Acid soil, NS = Non-calcareous soil, CV = Coefficient of variance, LSD = Least significant difference, SE± = Standard error of means, Exch.= Exchangeable

Table 5b. Effects of different levels of cow dung (CD) on the fertility of post harvest soils

Levels of CD	Available S (μg g^{-1})		Exch. Ca (Cmol kg^{-1})		Exch. Mg (Cmol kg^{-1})		Available Zn (μg g^{-1})		Available B (μg g^{-1})	
	AS	NS	AS	NS	AS	NS	AS	NS	AS	NS
CD_0	13.06d	13.00d	0.15b	2.10b	0.60b	1.20b	1.30b	0.80	0.33b	0.23b
CD_5	15.10c	15.19c	1.89a	6.95a	0.72b	3.39ab	1.64a	0.84	0.36b	0.29b
$CD_{7.5}$	18.35b	22.87b	2.15a	7.20a	0.97b	5.59a	1.75a	0.87	0.79a	0.36a
CD_{10}	22.90a	27.95a	2.43a	8.43a	1.84a	7.69a	1.83a	0.94	0.92a	0.47a
CV(%)	4.19	11.40	12.73	5.32	4.57	8.31	5.22	5.81	10.13	5.30
$LSD_{0.05}$	1.60	4.21	0.35	0.86	0.13	0.56	0.64	0.32	0.20	0.07
SE±	0.53	2.39	0.42	0.53	0.06	0.52	0.10	0.09	0.07	0.05

AS = Acid soil, NS = Non-calcareous soil, CV = Coefficient of variance, LSD = Least significant difference, SE±= Standard error of means, Exch.= Exchangeable

The organic matter (OM) content of the post-harvest soils significantly increased due to the application of cow dung in both soils as it contains higher amount of OM than any other organic additives of soil. Organic matter content of the acid soil ranged from 1.70 to 2.55% where as in the non-calcareous soil it ranged from 1.28 to 2.96%. The contents of total N, available P, exchangeable K, Ca, Mg, available S, Zn, B were significantly increased with the increased levels of CD up to 10 t ha^{-1} in both soils. All the nutrient contents except available P content were much higher in non-calcareous soil compared to acid soil. The highest values of the parameters were obtained from CD_{10} and the lowest from the initial soil (CD_0).

Conclusion

The present study revealed that the application of cow dung at different levels positively influenced all the characters studied. Cow dung application have been found to be increased the total N, available P, exchangeable K, Ca, Mg, available S, Zn and B contents in soils and biomass yield of stevia over the control significantly with the increased levels of CD up to its highest level in both soils. The parameters were increased with the advancement of growth period (60 DAP) and increased rate of CD up to 10 t ha^{-1}. Thus it can be advised that CD @ 10 t ha^{-1} might be applied to get maximum leaf biomass yield of stevia and for increasing the fertility of both soils.

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