ISSN: 2518-2021 (Print), 2415-4474 (Online) Fundam Appl Agric 2017, 2(3): 297-302

Fundamental and Applied Agriculture

Journal homepage:www.f2ffoundation.org/faa

Crop Science
ORIGINAL ARTICLE

Potassium requirement for leaf biomass yield and K nutrition of stevia

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A R T I C L E I N F O

Article history:

Received: 27 August 2017
Received in revised form: 15 September 2017
Accepted: 25 September 2017
Available online: 30 September 2017

Doi: 10.5455/faa.276431

Academic Editor: Esra Uçar Sözmen

Keywords:
Stevia
Leaf yield
K requirement
Critical K content

A B S T R A C T

Potassium (K) is an essential plant nutrient affecting most of the biochemical and physiological processes. It is classified as a macronutrient due to large quantities being taken up by plants during their life cycle. With the aim of establishing a scientific basis of K fertilization on the growth, leaf production, minimum K requirement and critical leaf K concentration of stevia, a pot experiment was carried out in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University. Six levels of K *viz.* 0 (K_0), 50 (K_{50}), 100 (K_{100}), 150 (K_{150}), 200 (K_{200}) and 250 (K_{250}) kg ha^{-1} were used in acid and non-calcareous soils. Plants were harvested at 60 days after planting (DAP). Results showed that K application significantly influenced the growth attributes, leaf yield and other parameters. Highest values of most of the parameters were obtained at 200 kg K ha^{-1}. Dry weight of stevia leaves at harvest was increased from 0.77-7.41g and 0.99-7.74g Pot^{-1} in acid and non-calcareous soils, respectively over control. Potassium content was directly proportional with the increased levels of K though uptake did not follow the same trend. Leaf critical K content was estimated to be *ca* 1.75 and 1.71% in the plants grown in acid and non-calcareous soils, respectively. The minimum K requirement for maximum leaf biomass production (80%) of stevia was also estimated to be *ca* 220 and 199 kg ha^{-1} grown in acid and non-calcareous soils, respectively. This study would contribute to improve K fertilization of stevia in various types of soil.

INTRODUCTION

Potassium (K) participates in many important functions in plants i.e., photosynthesis, translocation of photosynthates, protein synthesis, control of ionic balance, regulation of plant stomata and water use (Reddy et al. 2004), enzyme activation and osmoregulation (Mengel 2007). Out of all the mineral nutrients, K plays a particularly critical role in plant growth and it contributes greatly to the survival of plants under various biotic and abiotic stresses (Wang et al. 2013). Potassium performs important roles as plant nutrient to sustain high productivity and quality, equilibrium with other essential plant nutrients.

Stevia (*Stevia rebaudiana* Bertoni.) is a plant which was originated from South America (Paraguay and Brazil), belongs to the family Asteraceae. Stevioside is one of the active constituents which is available abundantly in the leaf of stevia (5-10% of dry weight basis) and is 300-350 times sweeter than sucrose (Zhang et al. 1999). Stevia acts as flavor enhancer and in medicinal field having hypoglycemic, oral contraceptive, cardiovascular and antimicrobial activity. The stevioside of stevia having least caloric value is recommended in various ailments particularly diabetes. It is also used to weight loss, digestive and skin problems. Literature survey revealed active constituents especially stevioside content in stevia plant is greatly depends on the package of practices for the cultivation and adoption of modern agro-techniques (Geuns 2003). It was also stated that proper cultivation of stevia will allow a 7-fold increase in production of sugar equivalent (Akatov et al. 2004). Studies conducted in India for stevia cultivation so far could suggest only few management approaches for improving productivity (Chalapathi et al. 1997b). Suitable soil for stevia cultivation has screened out (Zaman et al. 2015), N and S requirement and critical N and S content of stevia grown in two contrasting soils of Bangladesh has been reported by Zaman et al. (2016a and 2016b). Recently, PUE, critical P content and minimum

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P requirement of stevia was also reported by Zaman et al. (2017).

Bangladesh being an agro-based country could easily introduce this plant as an industrial crop like sugarcane, sugar beet, tea or coffee and can commercially be cultivated in its relatively high land, char land, home stead area etc. as it grows well in open space having regular sun light. For the insufficiency of adequate information regarding K requirement for stevia cultivation under the agro-climatic conditions of Bangladesh, there is a need to set up certain protocols for stevia cultivation in various soil conditions of our country. Therefore, the present investigation was undertaken to evaluate the effects of different K levels on the growth, leaf biomass yield, K content and its uptake, minimum K requirement and critical leaf K concentration of stevia under the environmental conditions of Bangladesh Agricultural University, Mymensingh. This finding will provide important information to the basic understanding of K requirement for the leaf biomass yield and K nutrition of stevia.

METHODOLOGY

A pot experiment was carried out to study the response of stevia plant by the application of different K levels. *In vitro* produced 45 days old stevia seedlings, collected from *brac* biotechnology laboratory, Joydebpur, Gazipur, were used as a test plant for the present study. Detailed materials and methods can be seen from Zaman et al. (2016). Two soils *viz*. acid and non-calcareous of contrasting physical and chemical properties were used for growing stevia (Zaman et al. 2015). The physical and chemical properties of soil were determined following standard procedure described by Page et al. (1982). One stevia seedling was planted in each pot with the basal application of N, P, S, Zn and B @ 250, 100, 30, 3 and 1 kg ha^{-1} from prilled urea, TSP, gypsum, zinc sulphate and boric acid, respectively (Zaman 2015). Six levels of K *viz*. 0 (K_0), 50 (K_{50}), 100 (K_{100}), 150 (K_{150}), 200 (K_{200}) and 250 (K_{250}) kg ha^{-1} were applied from MoP (Muriate of Potash). Nitrogen was applied as prilled urea in three equal installments, $1/3^{rd}$ during pot preparation, $1/3^{rd}$ at 15 days after planting (DAP) and $1/3^{rd}$ at 30 DAP. The experiment was laid out following completely randomized design (CRD) with three replications of each treatment. After harvesting the crop at 60 DAP, leaf samples were processed and stored. The K concentrations of stevia leaf were determined by flame emission spectrophotometer as outlined by Knudsen et al. (1982). Potassium uptake was calculated from K content and leaf dry yield. Potassium requirement and critical K content of stevia was estimated as described by Zaman et al. (2017). The results obtained were analyzed statistically using standard methods of analysis (Steel et al. 1997). The differences among the treatment means were compared by using Latin Square Design (LSD) (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Effects of different levels of potassium on various parameters of stevia have been presented under the following heads

Effects of K on plant height

From obtained results, it could be noticed that plant height of stevia at harvest (60 DAP) was significantly influenced with the increasing levels of K (Table 1). The treatment K_{200} recorded significantly highest plant height (94.7 and 97.0 cm in acid and non-calcareous soils, respectively) which was statistically identical with K_{150} and K_{250} but significantly different from others (K_{50} and K_{100}). The potassium levels of K_0 (control) recorded the shortest plant (67 and 61 cm in acid and non-calcareous soils, respectively). Potassium application at all levels increased plant height by 6.3 to 27.7 cm in acid soil and 16.0 to 36.0 cm in non-calcareous soil over control.

The result shows the restricting factors of the stevia growth occurs in the soil nutrient deficiency at low levels of K, but as increasing K levels soil nutrient raised and grew rapidly at K_{200}. After this level, the toxicity of K fertilizer might arise which restrict the growth of stevia. These results are in accordance with the findings of Singh et al. (2015) who reported the higher plant height (37.2 cm) of stevia @ 150 kg K ha^{-1}.

Table 1. Effects of different levels of K on the growth parameters of stevia at harvest

K level	Plant height (cm)		Branch number		Leaf number		Leaf area $plant^{-1}$ (cm^2)	
	AS	NS	AS	NS	AS	NS	AS	NS
K_0	67.0d	61.0c	3.0b	3.0b	45.0e	53.0e	238e	363c
K_{50}	73.3cd	77.0b	4.7b	4.0ab	70.0d	85.0d	452de	587c
K_{100}	77.7bcd	83.7ab	5.3ab	5.0ab	91.0cd	125.0c	618cd	937bc
K_{150}	84.7abc	85.3ab	7.3a	7.3a	115.0c	170.0b	776c	1413b
K_{200}	94.7a	97.0a	7.7a	8.3a	290.0a	315.0a	2066a	3216a
K_{250}	92.0ab	91.7ab	7.3a	7.7a	170.0b	190.0b	1175b	1630b
CV(%)	1.2	1.3	3.6	4.1	5.1	4.5	6	6
$LSD_{0.05}$	8.4	8.2	2.5	2.3	15.9	18.0	170	400
SE±	2.9	3.2	0.6	0.7	20.0	20.9	149	240

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

Increased plant height of stevia with nutrient levels of 40:20:30 kg N, P, K ha^{-1} have also been reported in sandy loam soils at Bangalore (Chalapathi et al. 1999). Kawatani et al. (1980) reported increased plant height, number of branches and leaves $plant^{-1}$ of stevia plant by application of higher levels of K fertilizers. Similarly, Sharma and Sharma (2010) also found the highest plant height (58.88 cm) at 100% recommended doses of NPK (125-75-60 kg ha^{-1}) compared to lower doses in cauliflower.

Effects of K on branch number

Branch number of stevia plant was significantly affected by the addition of K in both soils at 60 DAP (Table 1). The number of branches $plant^{-1}$ was significantly increased with the increased levels of K up to 200 kg ha^{-1} and then slowly decreased with further increase in K levels (250 kg ha^{-1}). Potassium application at all levels increased branch number by 56-154% in acid soil and 33 to 177% in non-calcareous soil. The highest number of branches plant^{-1} was observed at K_{200} which was significantly identical with K_{250} and K_{150} but statistically different from K_{100} and K_{50} in acid soil whereas identical results on branch number were observed with all the K levels except control in non-calcareous soil. Crop performance to a great extent is governed by the number of branches plant^{-1}. It is, therefore, imperative that if the number of branches plant^{-1} is higher, the numbers of leaves are expected to be higher; ultimately the leaf yield will also be higher. This finding is also similar with the results of Singh et al. (2015) who reported that number of branches plant^{-1} in stevia is highest at K @ 150 kg ha^{-1}. Sanap et al. (2010) reported that the number of branches, diameter of fruit, yield vine^{-1} and yield ha^{-1} were highest when 250 kg N, 50kg P_2O_5 and 100kg K_2O ha^{-1} was applied in bitter gourd.

Effects of K on leaf number

The number of leaves plant^{-1} would also substantiate the fact that increased number of leaves would contribute to the final yield of the plant. It is more appropriate for the crops like stevia in which only leaves are used for commercial product. The data on the number of leaves plant^{-1} were significantly influenced by different levels of K in both acid and non-calcareous soils at the time of harvest (Table 1). The number of leaves plant^{-1} was increased with the increased levels of K up to 200 kg ha^{-1} and then decreased with further addition (K_{250}). Potassium application at all levels increased the number of leaves by 25 to 245 in acid soil and 32 to 252 in non-calcareous soil over control. Maximum number of leaves was recorded with K_{200} which was statistically different from all other levels of K in both soils. Plants fertilized with K_{50} and K_{100} produced identical number of leaves in acid soil whereas K_{250} and K_{150} fertilized plants produced identical number of leaves in non-calcareous soil. The minimum number of leaves plant^{-1} was harvested from K control irrespective of soils.

The results of present study are in accordance with the findings of Singh et al. (2015) who found highest number of branches and leaves plant^{-1} of stevia with K @ 150 kg ha^{-1}. Increased number of leaves plant^{-1} with increased levels of N, P and K was also reported in Brazil (Rashid et al. 2013). Aladakatti et al. (2012) concluded that application of higher levels of K increased stevia plant height, number of branches plant^{-1} and number of leaves plant^{-1} resulting into higher leaf yield. Mantur (1988) also reported that higher plant height, number of branches, total dry matter accumulation, leaf area and leaf area index with a combination of 180:120:75 kg NPK ha^{-1} in China aster.

Effects of K on leaf area

Leaf area is an important growth index determining the capacity of plant to trap solar energy for photosynthesis and has marked influence on the growth and yield of plant. The total leaf area plant^{-1} at harvest was significantly influenced by different levels of K (Table 1). The results revealed that leaf area progressively increased with increasing levels of K application up to 200 kg ha^{-1} in both soils and then decreased with further addition of K. Like other yield attributes, the highest total leaf area plant^{-1} (2066 cm^2 in acid soil and 3216 cm^2 in non-calcareous soil) at harvest was measured from the plant receiving 200 kg K ha^{-1} which was significantly different from other levels of K. Second highest values (1175 cm^2 in acid soil and 1630 cm^2 in non-calcareous soil) were obtained from K_{250} which were significantly different from other levels in acid soil and identical to K_{150} and K_{100} in non-calcareous soil. The lowest leaf area was found from the control treatment which was identical with K_{50} irrespective of soils used. At harvest, K application at all levels increased leaf area by 90 to 768% in acid soil and 62 to 786% in non-calcareous soil was observed. Higher leaf area of stevia with higher K levels could be attributed to more number of branches and leaves plant^{-1} due to higher plant height. Khanom (2007) reported highest leaf area of stevia plant grown in non-calcareous soil applying chemical fertilizers. Tripathy et al. (1993) found that plant height and leaf area in spine gourd were greater with K_2O @ 60 kg ha^{-1}.

Table 2. Effects of different levels of K on leaf fresh and dry weight and yield increase of stevia leaves over control at harvest

K level	Leaf fresh weight (g plant^{-1})		Leaf dry weight (g plant^{-1})		Yield increase over control (%)	Yield increase over control (%)
	AS	NS	AS	NS	AS	NS
K_0	5.05e	5.99e	1.36e	1.61e	-	-
K_{50}	7.85de	9.53d	2.13de	2.60d	57	61
K_{100}	10.21cd	14.03c	2.78cd	3.78c	104	135
K_{150}	12.91c	19.08b	3.50c	5.14b	157	219
K_{200}	32.54a	34.70a	8.77a	9.35a	545	481
K_{250}	19.08b	21.32b	5.15b	5.74b	279	257
CV (%)	5.08	4.38	5.09	4.37	-	-
$LSD_{0.05}$	1.82	2.03	0.47	0.54	-	-
SE±	2.23	2.29	0.60	0.62	-	-

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

Effects of K on leaf fresh weight

The results presented in Table 2 showed that the fresh biomass yield of stevia leaves plant^{-1} at harvest was influenced by different levels of K fertilizer. The magnitude of such changes varied with treatments, being recorded highest fresh weight plant^{-1} (32.54g in acid soil and 34.70g in non-calcareous soil) at harvest was obtained from the plant fertilized with 200 kg K ha^{-1} which was significantly higher than other levels of K. Results revealed that leaf fresh weight significantly and progressively increased with the increased levels of K application up to 200 kg ha^{-1} in both soils and then decreased with further addition (K_{250}). As expected, the lowest values were obtained from the control treatment. Potassium application at all levels increased fresh weight at harvest by 2.80 to 27.49g plant^{-1} and 3.54 to 28.71g plant^{-1} in acid and non-calcareous soil, respectively. This finding is similar with the results of Rao and Subramanian (1991) who found increased fresh yield of tomato fruits with increased levels of potassium and highest fruit yield was reported at 150 kg K_2O ha^{-1}. Majumdar et al. (2000) observed that, increased levels of potassium had significant influence on the fruit yield of tomato and they obtained highest yield at 90 kg K_2O ha^{-1}.

Effects of K on leaf dry weight

Dry matter accumulation by the crop is another important growth parameter to be considered for determining the economic yield while assessing the effects of different treatments. Potassium at different levels showed significant influence on the dry matter production of stevia (Table 2). Results showed that leaf dry weight significantly and progressively increased with the increased levels of K application up to K_{200} in both soils and then decreased with further addition of K. The highest dry weight plant^{-1} (8.77g in acid soil and 9.35g in non-calcareous soil) at harvest was obtained from K_{200} which was significantly different from other levels of K. Second highest values (5.15g in acid soil and 5.74g in non-calcareous soil) were obtained from K_{250} which was statistically different from others in acid soil but identical with K_{150} in non-calcareous soil. The lowest values were obtained from the control treatment. Potassium application at all levels increased leaf dry yield at harvest by 57 to 545% and 61 to 481% in acid and non-calcareous soils, respectively over control. These results are in conformity with the findings of Chalapathi et al. (1997a and 1999) who reported that with the application of N, P and K at 60, 30 and 45 kg/ha, respectively, produced higher dry leaf yield with the simultaneous higher nutrient uptake by stevia plant. Our findings are also in agreement with that of Ojeniyi et al. (2007) who reported that application of N, P, K and animal manure increased the dry weight of tomato as compared to control. Hussain et al. (2014) found that application of 150 kg K_2O ha^{-1} promoted sugar beet top yield by 49.2% and fresh root yield by 45.0% over control treatment.

Leaf K content and uptake

The data on the K concentration and uptake by stevia leaves have been presented in Table 3. Both K content and uptake were significantly influenced by the application of K fertilizers. Potassium content of the leaf was increased with the increased levels of K irrespective of soils used. The highest K content (1.78% in acid soil and 1.82% in non-calcareous soil) was obtained when K was applied @ 250 kg ha^{-1} in both soils which was statistically identical with the K contents of the leaves of stevia plant fertilized with K_{150} and K_{200} but significantly different from other treatments. The lowest K content was obtained from K_0 in both soils. The K uptake varied from 14.96 to 144.71 mg Pot^{-1} in acid soil and 17.87 to 153.34 mg Pot^{-1} in non-calcareous soil. Potassium uptake as expected increased as K levels increased up to 200 kg ha^{-1} and then decreased with further addition (K_{250}). The lowest K uptake was observed in the control treatments of both soils. Higher nutrient uptake may be related to higher biomass yield. This may be due to the highest dry leaf yield harvested from that treatment (K_{200}). Shivaraj et al. (1997) found highest uptake of K by stevia leaf @ 45 kg K ha^{-1}. Leaf potassium concentrations of almond trees (*Prunus dulcis*) less than 0.5-0.6% appeared to limit leaf CO_2 exchange rate (Basile et al. 2003). They also found that potassium deficiency in almond affected the leaf photosynthetic capacity via biochemical limitations and not through an effect on stomatal conductance. According to Leigh and Johnston (1983), low nutrient content in the plant is a poor indicator of soil K availability.

Critical K concentration of stevia leaf

The critical K concentration in stevia leaf was estimated from the relative amount of leaf biomass to achieve 80% of the maximum production of stevia leaf. For both the soils, relative leaf biomass yield was plotted on the ordinate (Y axis) against the respective K concentration of stevia leaf on the abscissa (X axis) in Figure 1. The graph shows a regular but diminishing increase in yield with increasing concentrations of K in stevia leaf, except at the highest concentration where there was a slight reduction in yield. Increasing K concentrations were associated with increases in yield (Figure 1). We followed the "Critical nutrition concentration" concept advanced by Ulrich (1952) for plant to determine critical K concentration in stevia leaf. Critical values as used by Ulrich and Hills (1973) are determined from the relationship of nutrient concentration and relative yield at the time of sampling. The K concentration corresponding to the arbitrary point at 80% to achieve the maximum leaf biomass production was estimated by the fitted curve to be *ca* 1.75 and 1.71% in the leaves of stevia plants grown in acid and non-calcareous soils, respectively (Figure 1).

Table 3. Effects of different levels of K on its content and uptake by stevia leaf at harvest

K level	Potassium			
	Acid soil		Non-calcareous soil	
	Content (%)	Uptake (mg pot^{-1})	Content (%)	Uptake (mg pot^{-1})
K_0	1.10c	14.96d	1.11c	17.87e
K_{50}	1.11c	23.64d	1.13c	29.38e
K_{100}	1.46b	40.59c	1.49b	56.32d
K_{150}	1.53ab	53.55c	1.56ab	80.18c
K_{200}	1.65ab	144.71a	1.64ab	153.34a
K_{250}	1.78a	91.67b	1.82a	104.47b
CV (%)	1.60	5.92	1.60	5.12
$LSD_{0.05}$	0.16	7.80	0.16	8.91
SE±	0.07	10.92	0.07	11.30

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

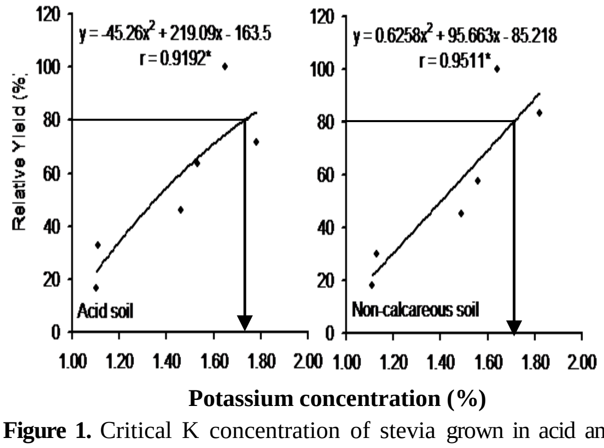

Figure 1. Critical K concentration of stevia grown in acid and non-calcareous soils. Values are the means of all treatments. **Correlated significantly at P<0.01.

Recently, Zaman et al. (2017) estimated critical P concentration for maximum leaf biomass production to be *ca* 0.19 and 0.30% in the leaves of stevia plants grown in acid and non-calcareous soils, respectively. Yin and Vyn (2004) estimated the critical leaf K concentration for maximum yield of soybeans to be 25.9 g kg^{-1}. Smith et al. (1985) showed that perennial ryegrass produced 98% of the maximum dry matter where K concentrations in the shoots were greater than 36 g kg^{-1}.

Potassium requirement of stevia plant

To determine the requirement of K to obtain 80% of maximum leaf biomass yield, the applied K was plotted on the X axis against the relative leaf biomass yield on the Y axis. From the fitted curve, the estimated amount of K for leaf biomass production of stevia grown in acid and non-calcareous soils to be *ca* 220 and 199 kg ha^{-1}, respectively (Figure 2).

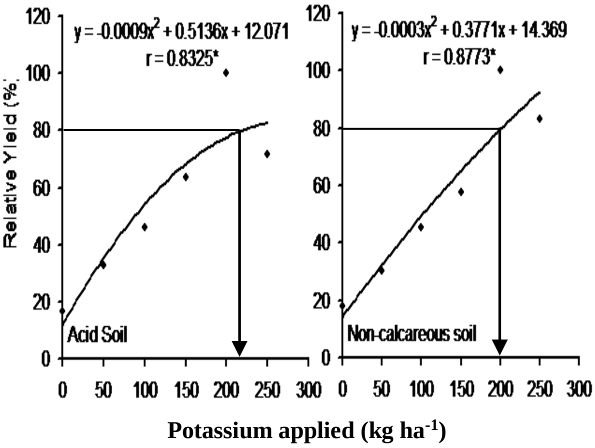

Figure 2. K requirement of stevia grown in acid and non-calcareous soils. Values are the means of all treatments. **Correlated significantly at P<0.01.

A crop's requirement for a specific nutrient is commonly defined as "the minimum content of that nutrient associated with the maximum yield" or "the minimum rate of intake of the nutrient associated with the maximum growth rate" (Loneragan 1968). Gobarah et al. (2011) reported that application of 114 kg K_2O ha^{-1} in sugar beet (*Beta vulgaris* L.) associated with significant increases in root and foliage weights and root dimensions, sucrose and purity percentages and sugar yields per hectare. El-Sarag and Moselhy (2013) showed that highest sugar beet yields (top, root and sugar/ha) were obtained by adding 140 kg K_2O ha^{-1}. Recently, N, P and S requirements of stevia were estimated to be 273, 109, 40 kg ha^{-1}, respectively in acid soil and 257, 45, 104 kg ha^{-1}, respectively in non-calcareous soil (Zaman et al. 2016a; Zaman et al. 2017; Zaman et al. 2016b).

CONCLUSION

It can be concluded that maximum stevia growth, yields and its components were resulted from 200 kg K ha^{-1}. The decreasing trends in these parameters with further increase beyond 200 kg K ha^{-1} indicated that higher doses of K could be detrimental for stevia and it should be avoided. Application of K @ 200 kg ha^{-1} gives highest leaf dry yield at harvest (545% in acid soil and 481% in non-calcareous soil). Same responses were found in case of K content and K uptake by stevia in both soils. The results suggested that the applications of K @ 220 kg ha^{-1} in acid soil and 199 kg ha^{-1} in non-calcareous soil in order to derive optimum growth, leaf biomass yield and K nutrition of the stevia plant would be useful under the agro-climatic conditions of Bangladesh Agricultural University.

ACKNOWLEDGEMENTS

The authors are thankful to Bangladesh Agricultural Research Council (BARC), Farmgate Dhaka for encouragement and providing financial support for this study.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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