



Stevia (Stevia rebaudiana Bertoni) responds to different levels of nitrogen and potassium fertilizers in loamy sand soil

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Abstract

A study was conducted in Al-Seba Reserve / Basra Governorate to study the effect of nitrogen and potassium fertilization on growth of stevia plants during 2018-2019 agricultural season, in pots. A sandy mixture of soil was used and two factors were studied: the first factor was urea fertilizer with five levels of nitrogen (N₀ 0, N₁ 100, N₂ 150, N₃ 200, and N₄ 250 kg / ha) and the second factor was potassium sulfate with three levels of potassium (K₀ 0, K₁ 75 and K₃ 150 kg / h) . The experiment was experimental factor using a complete randomized design (C.R.D) with three replications. The results showed a significant effect of adding nitrogen and potassium fertilizers and there interaction on: plant height, number of branches, leaf area index, and the treatment N₃K₂ gave the highest yield reached (1.27 tons. h⁻¹), and N₄K₂ recorded a highest content of Rebaudioside A (53.26 ppm).

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أستجابة الاستيفيا (*Stevia Rebaudiana Bertoni*) لمستويات مختلفة من السماد النتروجيني والبوتاسي في تربة مزيجية رملية.

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أجريت دراسة في محمية السبيبة / محافظة البصرة لدراسة تأثير التسميد النتروجيني والبوتاسي على نمو محصول الاستيفيا خلال الموسم الزراعي 2018-2019، داخل أصص . استخدمت تربة مزيجية رملية وأجريت دراسة عاملين: العامل الأول سماد اليوريا بخمس مستويات من النيتروجين (0 , 100 , 150 , 200 , 250 كغم/هكتار) والعامل الثاني سلفات البوتاسيوم بثلاث مستويات من البوتاسيوم (0 , 75 , 150 كغم/هكتار) صممت التجربة عاملية وباستخدام التصميم العشوائي الكامل (C.R.D) بثلاث مكررات. اظهرت النتائج التأثير المعنوي لإضافة السماد النتروجيني والبوتاسي والتداخل فيما بينهما على كل من: ارتفاع النبات، عدد الافرع بالنبات، دليل المساحة الورقية، واعطت المعاملة العاملية N3K2 اعلى حاصل بلغ (1.27 طن.هـ⁻¹)، كما سجلت المعاملة العاملية N4K2 اعلى محتوى من Rebaudioside A بلغ (53.26 جزء بالمليون).

*البحث مستل من رسالة الدكتوراه للباحث الأول

Introduction:

Stevia rebaudiana Bertoni, which originated from South America, is a bushy branched plant of the Asteraceae family (Gisieine et al., 2006). It is one of the 154 members of the genus Stevia and one of only two that produce sweet Steviol glycosides which has been being used in food items as a sweetener material (Soejarto et al., 1982). The sweetness of stevia comes from compounds known as

diterpene glycosides (Brandle et al., 1992; Geuns, 2003). Among 10 sweet glycosides in stevia, two are the most important, namely stevioside and rebaudioside A (SGs) which are found to be the maximum immediately before flowering (Singh and Rao, 2005). Unlike many other sources of artificial or natural sweeteners, Rebaudioside-A has the most desirable flavor profile and in comparison, to other sources of artificial and natural sweeteners, stevia-based

sweeteners do not have aftertaste bitterness (Yadav, 2011). Stevia is grown as a crop in many countries including Japan, China, India, Korea, USA, Canada, Russia, Tanzania, Brazil, Paraguay, and Canada. This plant has an exceptional sweetness power; its sweetness power is rated between 50-400 times sweeter than white sugar. Fertilizer application in the crop field plays an important role in increasing crop production. The main macronutrients present in inorganic fertilizers are nitrogen, phosphorus, and potassium that influence both vegetative and reproductive phases of plant growth. Stevia being a leafy and bushy plant may need plenty supply of nitrogen (Inugraha et al., 2014; Maniruzzaman et al., 2016). Potassium is an essential quality nutrient after nitrogen. Potassium is needed in plants for hardening of the stem through fiber production preventing plants from lodging and making plants disease and pest tolerant, Potassium is an essential plant nutrient affecting most of the biochemical and physiological processes in plants along with stevia (Amtmann *et al.*, 2008). In a crop field, the soil texture greatly affects soil reaction, nutrient availability, water holding capacity, soil porosity, air-water circulation, and soil density. For this reason, the soil texture is considered a determinant for crop selection as the agronomic practices, and crop growth is largely affected by this soil structure (Chakraborty and Mistri, 2015). Shock (1982) reported that with a sufficient supply of water, stevia growth changed due to the change in soil physical properties. Therefore, to improve the growth and quality of

stevia, the study was designed to find the best fertilizer combination under loamy sand soil.

Materials and methods:

A pot experimental conducted in the growing season 2018-2019 in a private farm 'the Seba semi-ponic farm' located in Basra governorate in Seba, south of Basra (N 30.358°, S 48.155°), to discover the influence of nitrogen and potassium fertilizers on yield and quality of Stevia (*Stevia Rebaudiana* Bertoni). The experimental design was in factorial randomized complete design (C. R. D) with three replicates. The soil used in this trial was tested before experimentation for monitoring the nutrient status of the soil. Soil samples were analyzed at the University of Basra-Central Laboratory - Agriculture College. The nutrient status is shown in (Table1). The pots were filled with 7 kg of soil and after that, all the pots were irrigated up to the field capacity. Experimental consisted of two factors: Nitrogen fertilizer with five levels (0, 100, 150, 200, 250 Kg N.ha⁻¹) its symbolized it (N0, N1, N2, N3, N4) and Potassium with three levels (0, 75, 150 kg K₂O.ha⁻¹) it symbolized it (K0, K1, K2) including control, nitrogen as urea (containing 46% N) and potassium as potassium sulfate (containing 42% K) (Maheshwar, 2005 and Aladakatti, 2011).

Stevia seeds from (Zargiah farm- Shiraz- Iran) were sown in trays having peat and perlite and germinated under controlled conditions in a greenhouse in the Seba semi-ponic farm. During the germination stage, the favourable temperature and humidity were maintained in the greenhouse. After germination, the favourable

temperature and humidity were also maintained in the greenhouse until the seedlings reached a height of 10 cm. after that seedling transplanted, the vigorous seedlings were uprooted carefully from the pots so that roots were at minimal damage and then transplanted into the pots (permanent place) on 15-12-2018 and 1-1-2019. At the age of six weeks, two seedlings were transplanted at the center of each pot. The transplanted seedlings were established 15 days after transplantation and then the weaker seedling was removed keeping the more vigorous one in the pot. Nitrogen fertilizer (urea)

was applied in two equal splits; the first one was 14 days after seedling transplantation and the second one was 30 days after the application of the first dose. Potassium was added to the soil in one dose along with the first split of nitrogen fertilizer. The full dose of phosphorous fertilizer was applied to the soil before seedling transplanting (Aladakatti, 2011). All plants received normal agricultural practices during the growing season such as irrigation, weed control, deflowering, and plant protection measures particularly protecting the crop from Fusarium and whitefly.

Table (1) Some chemical and physical characteristics of experimental soil of Al-Zubair location

| Characters | value | Unit |
|--|------------|---------------------|
| Soil PH | 7.8 | |
| Soil E.C. (1:1) | 4.70 | Des.m ⁻¹ |
| N available (NH ₄ ⁺ + NO ₃ ⁻) | 75 | |
| Phosphor | 3.30 | Mg.kg ⁻¹ |
| potassium | 0.127 | |
| Organic matter | 0.15 | % |
| clay | 20.13 | |
| silt | 21.54 | |
| Sand | 58.33 | % |
| Texture | Loamy sand | |

Growth parameters studied:

Plant height (cm):

Plant height was measured by measuring tape from the soil surface to the fully opened leaf at the top and expressed in centimeters.

Number of leaves plant⁻¹:

The number of fully opened leaves was recorded before harvesting the crop, that after collecting the branches.

Leaf area index:

Leaf area index was calculated using the equation of Watson (1952) as follows;

$$LAI = \frac{\text{Leaf area per plant (cm}^2\text{)}}{\text{Land area occupied by each plant (cm}^2\text{)}}$$

$$\text{Leaf area per plant (cm}^2\text{)} = \text{Number of leaves} \times \text{leave area}$$

Leaf area was calculated by taking an average area of three leaves per plant using the Image J program.

Numbers of branches plant⁻¹:

The number of branches was recorded before harvest about (90) days after transplanting. Total branches (primary and secondary) were counted and the average number of branches per experimental unit was calculated.

Yield parameters:

After harvesting the plants from each of the pots, the fresh weight of the plants was taken and recorded in gm.plant⁻¹. The fresh weight was considered as the yield of the grown stevia. This weight was also considered as the biomass yield per pot. The surface of the soil in the pot was calculated on which the plants grew in the pot. The measured fresh biomass and then converted to the yield (ton. ha⁻¹).

Dry leaves yield is the economic yield of stevia, which is using for the extraction of stevioside. The dry weight plant⁻¹ and then converted into dry weight per hectare based on the surface area of each of the pots and recorded in terms of ton. ha⁻¹.

Nitrogen Concentration (%):

Nitrogen was estimated through microkjeldhal digestion and distillation Method following Jackson (1973). Then the N was calculated in terms of percentage.

Potassium Concentration (%):

Potassium was estimated from the digestive solution by Flame photometer according to black (1965).

Stevioside and rebaudioside content (ppm)

Glycosides that estimated using the HPLC technique. The stevioside content in the leaves which estimated by HPLC analysis as described by Hashimoto et al., (1978). Samples were analyzed in the laboratories of the Ministry of Science and Technology, Baghdad, Iraq.

Data were statistically analyzed using Genstat statistical program version 12, the differences between means were compared by the least significant difference (L.S.D) in the probability level 0.05 (Gomez and Gomez, 1984).

Results and discussion:

Plant height (cm):

Results showing the effect of nitrogen on plant height that presented in table (2). It was observed that there were significant differences between nitrogen and potassium fertilizers levels on plant height, the treatment N4 gave the highest plant height (48.33cm) showing significant differences with other treatments and increase in height of 39.1% over that of the control treatment. Nitrogen has a functional role in cell division and elongation, contributing to increase plant height which intern leads to a positive effect on plant height, plant height increased significantly with increasing the N fertilizer levels (Hassanen et al, 2016 and Inugraha et al., 2014).

Potassium application has also resulted in an increase in plant height which can be seen in (Table 2)and (Fig 1) the treatments K2 and K1 gave the heights plant height recording (42.2and 40.53 cm) high plants respectively, but these two treatments showed significant differences when compared with that of the control treatment which recorded the lowest plant height

(31.33cm). Potassium had a positive effect on increasing plant height, which may be attributed to its crucial function by enhancing photosynthesis and promoting both meristemic cell division and elongation. Such happens through an ideal occurring an ideal expansion of the cell wall that is essential for the division process, (Reddy et al. 2004 and Mengel and Kirkby, 2007). Results of this study agree well

with those of previous workers (Maheshwar, 2005; Ahmed et al, 2011; Hassanain et al, 2016). The significant interaction effect of nitrogen and potassium levels was also noticed in respect of plant height Fig (1) The impact of interaction revealed that combination treatment N4K1 had the highest plant height (55.33 cm) which was 81.7% more than the lower plant height values of the control treatment N0K0 (27.00 cm).

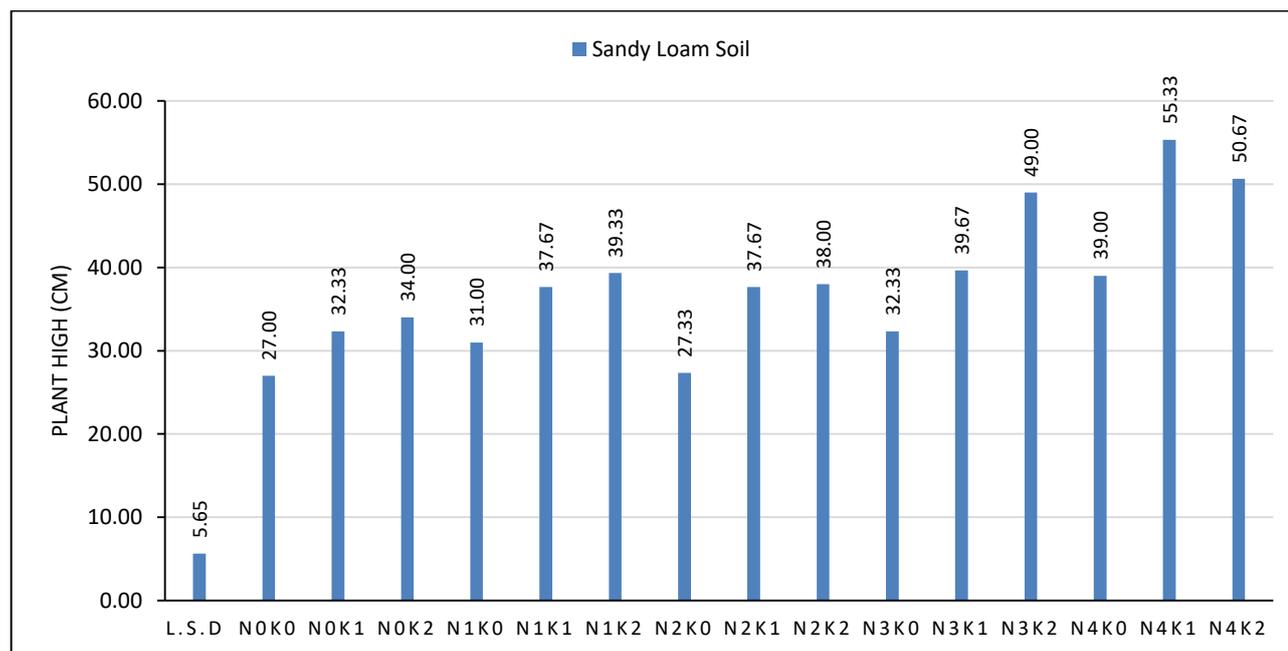


Figure 1 - Interaction Effect for different levels of (N & K) fertilizers on plant height (cm) of Stevia in Sandy Loam Soil

Numbers of branches plant⁻¹:

Different nitrogen and potassium levels influence positively the number of branches per plant (Table 2). Treatment N3 showed the highest number of branches 6.46 compare with other treatment, control treatment recorded the smaller number of branches 2.20. The availability of nitrogen at the beginning of plant growth encourages the growth and emergence of primary and secondary branches by supporting the growth buds and extending the duration of their production (Akbari et al, 2018). These results are in agreement with the finding of

(Aladakatti et al., 2012). They stated that increasing N levels increased the number of branches per plant.

potassium fertilizer levels, results indicated the significant effect of increased potassium levels on the number of branches soil treatment K2 recorded the highest number of branches (4.43) without significant differences with K1 which recorded (3.92),(Table 2) while these two treatments recorded the highest rate for this trait compared to the control treatment. This may be due to the positive effect in increasing the height of the plant and the number of nodes of the stem

by encouraging it to grow the tissue and improve the absorption of nutrients, as this was reflected in the increase in the number of vegetative branches of the plant and the growth of the plant in general. Karron *et al*, (1995). This finding agrees with Maniruzzaman *et al*, (2016) who pointed to the function of potassium in increasing the number of branches per plant.

The interaction effect of nitrogen and potassium on the number of branches was non-significant (Table 2), the control treatment recorded the lowest numerical value (1.11 and 1.68). This result is similar to the findings of (Aladakatti, 2011).

Number of Leaf per Plant:

Results showing the positive effect of nitrogen on the number of leaves. Plant⁻¹, which presented in table (2). It was observed that there were significant differences between nitrogen and potassium fertilizers levels on the number of leaves. Increasing nitrogen levels from 100 kg N ha⁻¹ to 200 kg N ha⁻¹ cause a significant increase in this parameter about 55.08% (from 33.89 to 52.56), while the control treatment gave the lowest number of leaf per plant (24.33). Increase leaf number per plant due to increasing nitrogen levels Kawatani, (1977). These results are in agreement with many reports this indicated an increase in the number of leaf per plant by increasing the level of nitrogen (Hassanain *et al*, 2016). Potassium application has taken a similar trend to what occurred with the application of nitrogen that lead to an increase in number of leaf per plant which can be seen in the table (2), treatments K1 and K2

recorded the maximum leaf number plant⁻¹ (42.67 and 40.2) respectively compared with control treatment which gave the lowest leaf number per plant (34.67), kawatani (1980), indicated to the positive effect of potassium fertilizer on leaf number per plant.

No Significant interactive effect was found between nitrogen and potassium levels on the number of leaves per plant. Table (2). **Leaf area index:**

Results showing the effect of nitrogen and potassium on plant height, which presented in Table (3). It was observed that there were significant differences between nitrogen fertilizer levels on leaf area index. It was observed from the results of Table (3), significant superiority of nitrogen fertilizer on leaf area index. N3 treatment gave the highest leaf area index 1.86 compared with other treatments, meanwhile, the control treatment recorded the smallest leaf area index 0.58. Results of this study agree well with those of previous workers Maheshwar, (2005); Aladakatti, (2011) indicated the significant impact of nitrogen fertilizer on leaf area index compared with control treatment or low levels of nitrogen fertilizer.

potassium fertilizer levels affect positively on leaf area index treatment K2 gave the highest leaf area index 1.46 with significant impact compared with k1 (1.19), while the control treatment recorded the lowest leaf area index (0.91). (Table2).

Potassium participates in metabolism processes, including regulating respiration through stomata, enzymatic activity in the formation of starch,

increasing resistance to drought and disease, therefore, have an impact on the growth and translocation of photosynthetic products between plant tissues which are reflected on leaf area per plant and then influence on leaf area index. (Inugraha, 2014). These results are following the finding of (Khanom, 2007).

The interaction between nitrogen and potassium on the leaf area index was significant in (Table

3) and (Fig. 2) results indicated the superiority of combination treatment N3 K2 (2.25) compared with other combination treatments. Control combination treatments the lowest leaf area index (0.31). These results are not consistent with (Inugraha *et al*, 2014 and Aladakatti, 2012) They indicated that there were no significant differences between nitrogen and potassium interaction.

Table 2 - Means of Plant Hight (cm), Number of Branches.Plant⁻¹, Number of leaves of Stevia Rebaudiana as influenced by different levels of Nitrogen and Potassium and their interaction

| | | Plant High (cm) | | | |
|-----------|------------|------------------------------|----------|-----------|-------|
| | | K (kg/ha) | | | |
| N (kg/ha) | | 0 kg/ha | 75 kg/ha | 150 kg/ha | Mean |
| control | 0 kg/ha | 27.00 | 32.33 | 34.00 | 31.11 |
| | 100 kg/ha | 31.00 | 37.67 | 39.33 | 36.00 |
| | 150 kg/ha | 27.33 | 37.67 | 38.00 | 34.33 |
| | 200 kg/ha | 32.33 | 39.67 | 49.00 | 40.33 |
| | 250 kg/ha | 39.00 | 55.33 | 50.67 | 48.33 |
| | Mean | 31.33 | 40.53 | 42.20 | |
| | L.S.D (%5) | A | B | AB | |
| | | 3.260 | 2.525 | 5.646 | |
| | | Number of Branches per Plant | | | |
| | | 0 kg/ha | 75 kg/ha | 150 kg/ha | Mean |
| control | 0 kg/ha | 1.68 | 2.24 | 2.68 | 2.20 |
| | 100 kg/ha | 2.51 | 2.78 | 3.90 | 3.07 |
| | 150 kg/ha | 3.01 | 4.46 | 5.00 | 4.16 |
| | 200 kg/ha | 5.47 | 6.24 | 7.68 | 6.46 |
| | 250 kg/ha | 4.35 | 3.90 | 2.89 | 3.71 |
| | Mean | 3.40 | 3.92 | 4.43 | |
| | L.S.D (%5) | A | B | AB | |
| | | 1.042 | 0.807 | ns | |
| | | Number of leaves | | | |
| | | 0 kg/ha | 75 kg/ha | 150 kg/ha | Mean |
| control | 0 kg/ha | 19.00 | 24.33 | 29.67 | 24.33 |
| | 100 kg/ha | 27.67 | 31.33 | 42.67 | 33.89 |
| | 150 kg/ha | 33.67 | 47.33 | 51.67 | 44.22 |
| | 200 kg/ha | 48.33 | 53.33 | 56.00 | 52.56 |
| | 250 kg/ha | 44.67 | 44.67 | 33.33 | 40.89 |
| | Mean | 34.67 | 40.20 | 42.67 | |
| | L.S.D (%5) | A | B | AB | |
| | | 7.374 | 5.712 | ns | |

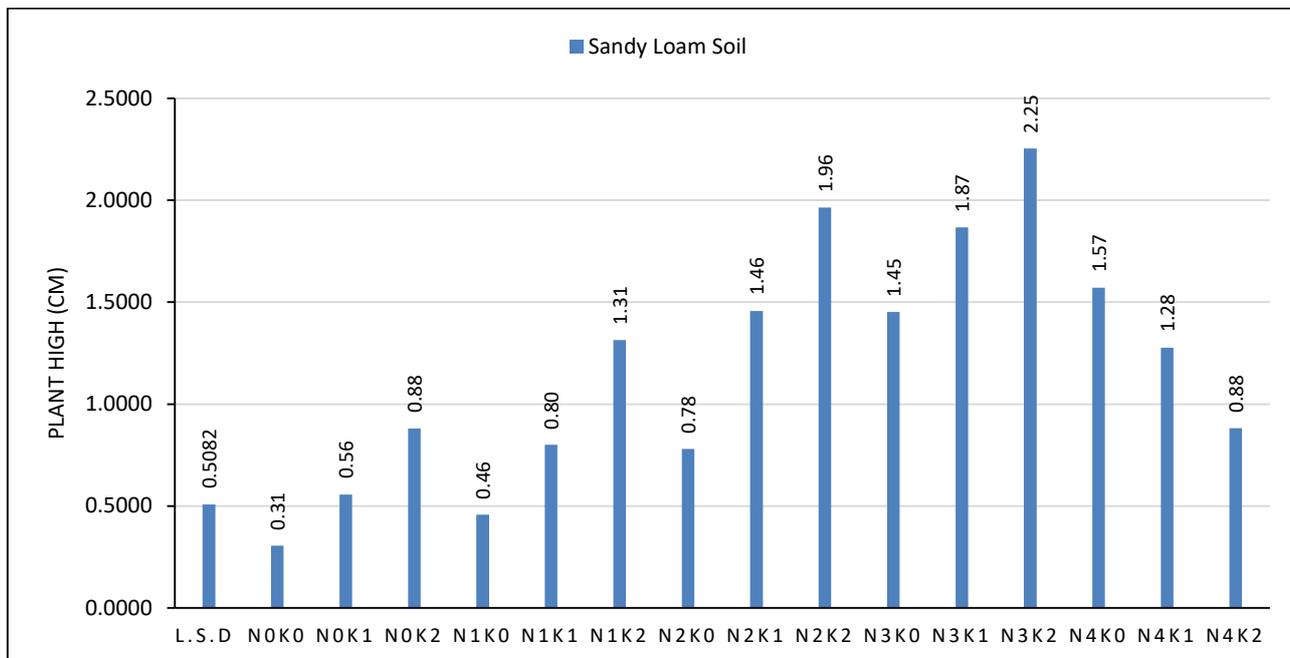


Figure 2 - Interaction Effect for different levels of (N & K) fertilizers on Leaf Area Index (LAI) of Stevia in Sandy Loam Soil

Total Yield ton.ha⁻¹:

High nitrogen levels contribute to the accumulation of dry matter and thus increased yields, this is important for the ability of the photosynthesis process and may support other synthetic processes during the development sequence. Rashid *et al.*, (2013).

The highest crop yield Table (3) was obtained from the nitrogen level of 200 kg.ha⁻¹ followed by the level 150 kg. ha⁻¹ recorded (1.03 and 0.80 kg. ha⁻¹) respectively, while the lowest crop yield was obtained from the control treatment (0.47 t.ha⁻¹). The outcomes are in agreement with Uçar *et al.*, (2018) which indicated the significant impact of nitrogen fertilizer on crop yield.

The function of potassium in plant growth must not be neglected, It would not only result in reducing growth parameters but would also change the translocation of the photosynthetic product (biomass) within plant tissue Redday and Zhoa,(2005) as a result potassium fertilizer

a significant effect on crop yield, potassium played an important role in many physiological and biochemical processes and has a positive effect on the movement and transfer of substances such as proteins and activated many enzymes, Helgi and Rolfe,(2005) and Havlin *et al.*,(2005). Results in (Table 3) indicated the significant impact of potassium on crop yield, the maximum yield obtained from K2 treatment (0.902 ton.ha⁻¹) as compared to other treatments, the lowest yield obtained from the control treatment (0.55 ton.ha⁻¹).

The interaction effects due to nitrogen and potassium were found significantly.(Table 3) and (Fig 3), shows the superiority of combination treatment N3 K2 (1. 28 t.ha⁻¹) as compared to other combination treatments, control combination treatment obtained the lowest yield (0. 22t.ha⁻¹). Macronutrient supplements that increase growth and enzymatic activity should also play a significant role in increasing the yield

components of stevia plants Aladakatti et al., (2012) Interaction between nitrogen and potassium fertilizer has a positive effect on growth parameters and yield. (Inugraha *et al.*, 2014). The present study finding conforms to the

finding of (Wiedenhoeft, 2006 and Rashid 2019). Were indicated to the significant effect of interaction between nitrogen and potassium on yield.

Table 3 - Means of Leaf Area Index (LAI) and Crop Yield t.ha⁻¹ of Stevia Rebaudiana as influenced by different levels of Nitrogen and Potassium and their interaction

| Leaf Area Index (LAI) | | | | |
|-----------------------|-----------|----------|-----------|------|
| N (kg/ha) | K (kg/ha) | | | Mean |
| | 0 kg/ha | 75 kg/ha | 150 kg/ha | |
| control 0 kg/ha | 0.31 | 0.56 | 0.88 | 0.58 |
| 100 kg/ha | 0.46 | 0.80 | 1.31 | 0.86 |
| 150 kg/ha | 0.78 | 1.46 | 1.96 | 1.40 |
| 200 kg/ha | 1.45 | 1.87 | 2.25 | 1.86 |
| 250 kg/ha | 1.57 | 1.28 | 0.88 | 1.24 |
| Mean | 0.91 | 1.19 | 1.46 | |
| L.S.D (%5) | A | B | AB | |
| | 0.293 | 0.227 | 0.508 | |

| Crop Yield t/ha | | | | |
|-------------------|-----------|----------|-----------|------|
| | K (kg/ha) | | | Mean |
| | 0 kg/ha | 75 kg/ha | 150 kg/ha | |
| control 0 kg/ha | 0.22 | 0.47 | 0.73 | 0.47 |
| 100 kg/ha | 0.31 | 0.60 | 0.80 | 0.57 |
| 150 kg/ha | 0.51 | 0.81 | 1.10 | 0.80 |
| 200 kg/ha | 0.80 | 1.02 | 1.27 | 1.03 |
| 250 kg/ha | 0.92 | 0.70 | 0.58 | 0.73 |
| Mean | 0.55 | 0.72 | 0.90 | |
| L.S.D (%5) | A | B | AB | |
| | 0.18 | 0.14 | 0.32 | |

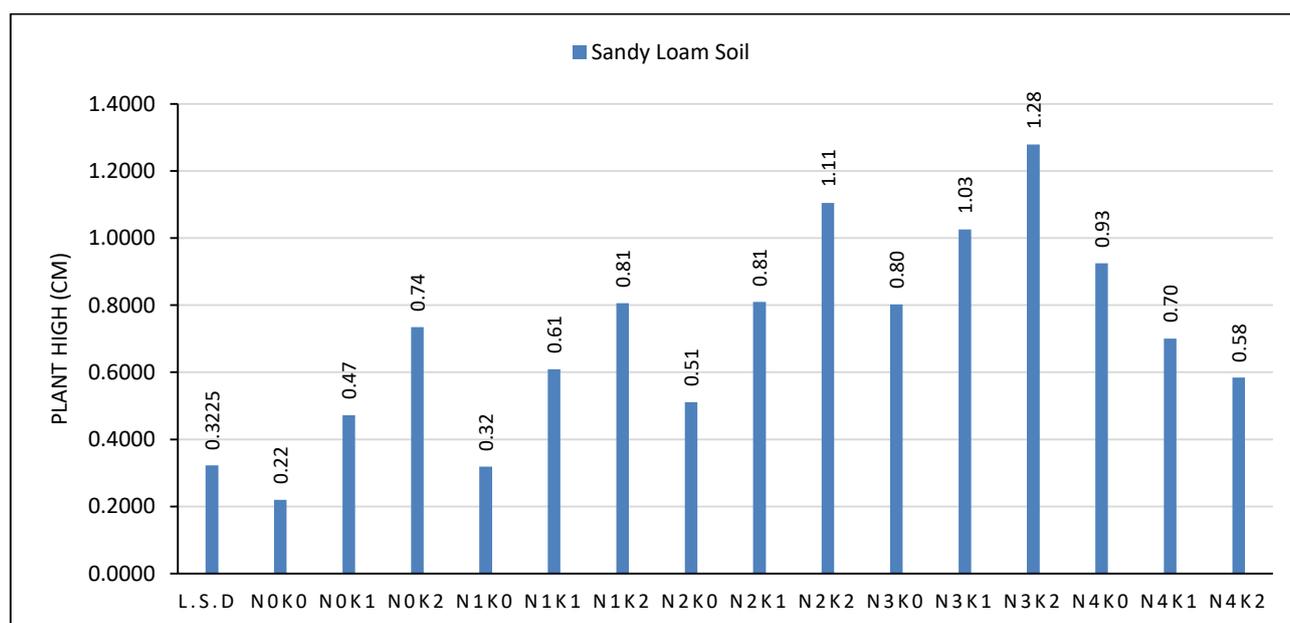


Figure 3 - Interaction Effect for different levels of (N & K) fertilizers on Crop Yield t/ha of Stevia in Sandy Loam Soil

Effect of Nitrogen and Potassium fertilization and their interaction on Nitrogen concentration (%):

Nitrogen concentration significantly increased in stevia dry leaves by increasing nitrogen fertilizer levels, results in Table (4), indicated that significant effect on nitrogen fertilizer on nitrogen content in dry leaves, maximum nitrogen content in the leaves was obtained from (250 kg.ha⁻¹) represented in N4 treatment (5.72%) as compared to other treatments, while control treatment obtained the lowest nitrogen concentration of 4.11%. Increased nitrogen absorption and content may be due to increased plant growth, Higher nutrient uptake was attributed to higher N fertilizer application. Nasrin, (2008). This finding in harmony with Hassanain *et al.*, (2016) was indicated to the higher content of nutrients in the stevia plant was attributed to the higher availability of nutrients in the root zone.

Potassium has an important role in increasing nitrogen absorption, increasing potassium fertilizer levels led to increasing nitrogen concentration in the dry leaf of stevia. Results in (Table 4), illustrated the significant effect of potassium on nitrogen absorption and concentration in dry leaves. the result revealed the superiority of the K2 treatment which achieved maximum nitrogen percentage (5.04%) compared with other treatments while the control treatment (K0) achieved the lowest nitrogen concentration (4.53%). the finding above is in harmony with the finding Hassanain *et al.*, (2016) were reported.

Higher NPK content recorded with higher NPK nutrients availability. They also indicated to the high nitrogen concentrations in leaf tissues was induced by the continuous supply of K⁺ from the available K⁺ in the soil and plant. The high absorption of nitrogen and potassium by the plant, which attributed to the high application of potassium.

No significant interaction effects between nitrogen and potassium levels were found on the nitrogen concentration in dry leaves.

Effect of Nitrogen and Potassium fertilization and their interaction on Potassium concentration (%):

Results in (Table 4) the results indicated that no significant differences observing between the levels of nitrogen. The control treatment recorded the lowest numerical value (3.82%).

Potassium plays a key role as a plant nutrient in sustaining high productivity and quality and maintaining the balance with other essential plant nutrients, Potassium improved cell permeability, which, in effect, helps the elements to pass rapidly to the root cells as a result minerals uptake increased, Inugraha *et al.*, (2014).

The significant impact of increasing potassium levels on potassium concentration in dry leaves of stevia results in (Table 4) revealed that K2 treatment obtained the maximum potassium concentration (4.92%) as compared to other levels while control treatment obtained the lowest potassium concentration (3.85%). Potassium absorption increase as expected with increasing levels of potassium fertilizer

(Maniruzzaman *et al.*, 2016). The results above are in agreement with the findings of Maniruzzaman *et al.*, (2016) Benhmimou *et al.*, (2018) refer to the increased potassium concentration according to increasing potassium levels. There was no significant interaction

between Nitrogen and Potassium levels on potassium concentration in dry leaves of stevia (Table 4). There are numerical differences between the combination treatments; control combination treatment recorded the lowest value (3.46%).

Table 4 - Means of Nitrogen percentage %N, Potassium percentage %K, Rebaudioside A (ppm) of stevia rebaudiana as influenced by different levels of Nitrogen and Potassium and their interaction

| | | Nitrogen percentage %N | | | |
|-----------|------------|-------------------------|----------|-----------|-------|
| | | K (kg/ha) | | | |
| N (kg/ha) | | 0 kg/ha | 75 kg/ha | 150 kg/ha | Mean |
| control | 0 kg/ha | 3.73 | 4.20 | 4.39 | 4.11 |
| | 100 kg/ha | 4.36 | 4.62 | 4.67 | 4.55 |
| | 150 kg/ha | 4.57 | 4.60 | 4.71 | 4.63 |
| | 200 kg/ha | 4.76 | 4.90 | 4.99 | 4.88 |
| | 250 kg/ha | 5.23 | 5.51 | 6.44 | 5.72 |
| | Mean | 4.53 | 4.76 | 5.04 | |
| | L.S.D (%5) | A | B | AB | |
| | | 0.470 | 0.364 | ns | |
| | | Potassium percentage %K | | | |
| N (kg/ha) | | 0 kg/ha | 75 kg/ha | 150 kg/ha | Mean |
| control | 0 kg/ha | 3.46 | 3.88 | 4.13 | 3.82 |
| | 100 kg/ha | 4.21 | 4.81 | 5.22 | 4.75 |
| | 150 kg/ha | 3.87 | 4.40 | 4.84 | 4.37 |
| | 200 kg/ha | 4.06 | 4.36 | 5.33 | 4.58 |
| | 250 kg/ha | 3.69 | 4.21 | 5.11 | 4.33 |
| | Mean | 3.86 | 4.33 | 4.93 | |
| | L.S.D (%5) | A | B | AB | |
| | | ns | 0.530 | ns | |
| | | Rebaudioside A, ppm | | | |
| N (kg/ha) | | 0 kg/ha | 75 kg/ha | 150 kg/ha | Mean |
| control | 0 kg/ha | 6.97 | 7.02 | 7.21 | 7.07 |
| | 100 kg/ha | 6.50 | 14.32 | 21.36 | 14.06 |
| | 150 kg/ha | 16.80 | 19.99 | 20.05 | 18.95 |
| | 200 kg/ha | 20.64 | 38.84 | 39.13 | 32.87 |
| | 250 kg/ha | 27.83 | 33.33 | 53.26 | 38.14 |
| | Mean | 15.75 | 22.70 | 28.20 | |
| | L.S.D (%5) | A | B | AB | |
| | | 2.611 | 2.023 | 4.523 | |

Effect of nitrogen and potassium fertilization and their interaction on Rebaudioside content:

Rebaudioside increases gradually because of increasing fertilizer levels (nitrogen and potassium). Results indicated the significant

effect of nitrogen, potassium, and their interaction on Rebaudioside content.

According to the nitrogen, impact in Table (4) results indicated the superiority of N4 treatment, which recorded the highest Rebaudioside content (38.14ppm) as compared with control treatment, which recorded the lowest rebaudioside content (7.07ppm). Many studies referred to the positive influence of nitrogen fertilizer on Rebaudioside accumulation in stevia leaves. Uçar *et al.*, (2018). The significant effect of potassium fertilizer on Rebaudioside content was shown in table (4) potassium fertilizer showed a significant impact on rebaudioside content K2 treatment recorded (28.2ppm) as compared with K1 (22.70) as well as the control treatment recorded (15.75ppm).

These findings are following the finding. Aladakatti *et al.*, (2012).

As seen in Table (4), Fig (4) the interaction between nitrogen and potassium has shown significant superiority. As seen in Tables (4) the interaction between nitrogen and potassium has shown significant superiority, combination treatment N4K2 recorded the highest Rebaudioside content (53.26ppm) as compared with the control treatment recorded the lowest Rebaudioside content (6.50ppm). These results are in harmony with the finding of Mostafa, (2019) indicated the positive relationship between increasing fertilizers rate and increase Rebaudioside content.

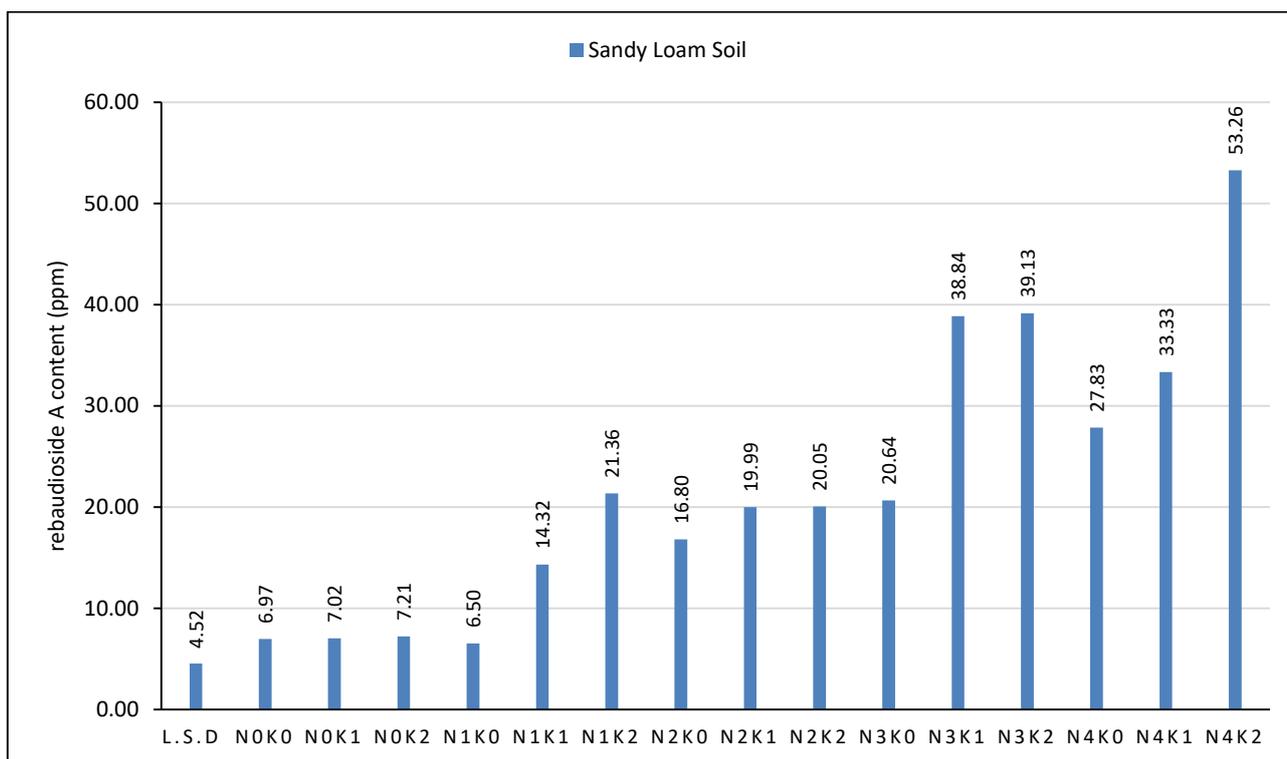


Figure 4 - Interaction Effect of different levels for (Nitrogen & Potassium) fertilizers on plant Rebaudioside A content (ppm) in Sandy Loam Soil

References:

- Ahmed B, Hossain M, Islam R, Saha A.k. and Mandal, A. (2011). A review on natural sweetener plant – Stevia having medicinal and commercial importance. *Agron Glas* 1: 75-92.
- Akbari, F., Arminian, A., Kahrizi, D., Fazeli, A., and Ghaheri, M. (2018). Effect of nitrogen sources on gene expression of *Stevia rebaudiana* (Bertoni) under in vitro conditions. *Cellular and Molecular Biology*, 64(2), 11-16.
- Aladakatti, Y. R., (2011) Response of *Stevia rebaudiana* Bertoni.) to irrigation schedule, planting geometry and nutrient levels, doctoral diss., Univ of Agric Sci, Dept of Agron, Dharwad.
- Aladakatti, Y. R., Palled, Y. B., Chetti, M. B., Halikatti, S. I., Alagundagi, S. C., Patil, P. L., and Janawade, A. D. (2012). Effect of nitrogen, phosphorus, and potassium levels on growth and yield of stevia (*Stevia rebaudiana* Bertoni). *Karnataka J. Agric. Sci*, 25(1), 25-29.
- Amtmann, A., Troufflard, S., Armengaud, P. (2008). The effect of potassium nutrition on pest and disease resistance in plants *Physiologia Plantarum*. Aug; 133(4):682-691. And Self-Incompatibility Mechanism of Genus *Stevia*.
- Benhmimou, A., Ibriz, M., Douaik, A., Lage, M., AlFaiz C., Chaouqi, S., Zouahri, A., (2018). Effect of NPK Fertilization on the Growth, Yield, Quality and Mineral Nutrition of New Sweet Plant in Morocco (*Stevia rebaudiana* Bertoni). *American Journal of Biology and Life Sciences*. Vol. 6, No. 3, pp. 36-43.
- Black, C. A. (1965). *Methods of Soil Analysis Part 1 and 2*. American Society of Agronomy, Inc.; USA.
- Brandle, J. E., and Rosa, N. (1992). Heritability for yield, leaf: stem ratio, and stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*. *Canadian Journal of Plant Science*, 72(4), 1263-1266.
- Chakraborty, K., and Mistri, B. (2015). Soil Fertility and Its' Impact on Agricultural Productivity: A Study in Sapor Mouza, Burdwan-I CD Block, West Bengal. *International Journal of Humanities and Social Science Studies (IJHSSS)*. 2(3), 196-206.
- Geuns J. (2003). Stevioside. *Phytochemistry*, 64: 913–921.
- Giseine, E.C., H.A. Abdol, C.A. Caudio, A.F. Leticia, T.Gilson, H.T Mirian, Wilson E.F and B.B. Roberto. (2006). Investigation of the tolerability of oral Stevioside in Brazilian hyperlipidemic Patients. *Brazilian Archives of Biol. and Tech., An International*, 49(4):583-587.
- Giuffré, L., Giardina, E., Ciarlo, E., Ríos, P., and Vella, L. (2015). A Study on the Effect of Soil Amendments and Environmental Conditions of *Stevia Rebaudiana* In Urban Soils of Buenos Aires, Argentina. *Current Agriculture Research Journal*, 3(1), 07-13. doi: <http://dx.doi.org/10.12944/CARJ.3.1.02>
- Gomez, K. A., and Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & Sons.
- Hashimoto, Y., Moriyasu, M., Nakamura, S., Ishiguro, S. and Komuro, M., (1978), High performance liquid chromatographic determination of *Stevia* components on a hydrophilic packed column. *J. Chromator*, 161: 403-405.
- Hassanain, M & Osman, A. & Sewedan, E. and Kotb, Sh.. (2016). Effects of Nitrogen fertilizer, humic acid foliar spraying, and their interactions on stevia (*stevia rebaudiana* l.) under reclaimed soil conditions in Egypt. *J.Agric.and Env.Sci.Dam.Univ.*, Egypt. 1515.
- Havlin, J. L. (2005). *Soil fertility and fertilizers: An introduction to nutrient management (7th ed.)*. Upper Saddle River, NJ: Pearson/Prentice Hall.
- Helgi, O. and S. A. Rolfe, (2005). *The Physiological of Flowering Plant*. 4th Ed., Camb. Univ. Press, Camb. UK., 100-106.
- Inugraha, M., Maghfoer, D., & Widaryanto, E. (2014). Response of *Stevia rebaudiana* Bertoni M) to nitrogen and potassium Fertilization. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 7(10), 47-55.

- Jia, G. N., (1984), An experiment on the cultivation of *Stevia rebaudiana* (Chinese). *Shanxi Agricultural Science*, 20 (1): p. 20-21.
- Karron, J. D., Thumser, N. N., Tucker, R., and Hossenauer, A. J. (1995). The influence of population density on outcrossing rates in *Mimulus ringens*. *Heredity*, 75(2), 175-180.
- Kawatani, T., Kaneki, Y., and Tanabe, T. (1977). On the cultivation of Kaa-hee (*Stevia rebaudiana* (Bert.)). *Japanese J. Tropical Agri.* 20, 137–142.
- Kawatani, T., Kaneyi, Y., Tanabe, T., and Takahashi, T. (1980). On the cultivation of Kaa-He- E (*Stevia rebaudiana* Bert.).VI. Response of to potassium fertilization rates and to the three major elements of fertilizer. *Nettai Nogyo*, 24, 105–112.
- Khanom, S. (2007). Growth, leaf yield and nutrient uptake by stevia as influenced by organic and chemical fertilizers grown on various types of soil (Doctoral dissertation, MS Thesis, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh).
- Maheshwar HM (2005). Effect of different levels of nitrogen and dates of planting on growth and yield of stevia (*Stevia rebaudiana*). M. Sc Thesis. Department of horticulture, University of agricultural sciences, Dharwad, Karnataka, India, 100p.
- Maniruzzaman M, MAH Chowdhury, KM Mohiuddin and T Chowdhury, (2016). Nitrogen requirement and critical N content of stevia grown in two contrasting soils of Bangladesh. *Res. Agric. Livest. Fish.* 3 (1): 87-97.
- Mengel, K., & Kirkby, E. A. (2007). Principles of plant nutrition 4th Edition international potash Ins. Bern, Switzerland, 687.
- Mostafa, H. S. (2019). Impact of NPK fertilization and lithovit rates on growth, yield components and chemical constituents of *Stevia rebaudiana* Bert. *Plant. Sciences*, 9(02), 412-420.
- Nasrin D, (2008). Effect of nitrogen on the growth, yield and nutrient uptake by stevia. MS Thesis, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh.
- Rashid Z, Rashid M, Inamullah S, Rasool S and Ah Bahar F (2013). Effect of different levels of farmyard manure and nitrogen on the yield and nitrogen uptake by stevia (*Stevia rebaudiana* Bertoni). *Afr. J. Agric. Res* 8: 3941-3945.
- Reddy AR, Chaitanya KV, Vivekanandan M. (2004). Drought- induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol*, 161:1189- 1202.
- Reddy, K. R. and D. Zhao, (2005), Interactive effect of elevated CO₂ and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton. *Field Crops Res* 94, 201-213.
- Shock, C.C. (1982). Experimental cultivation of Rebaudi's stevia in California. *Agron. Prog. Rep* 122: 250-258.
- Singh, S. D. and Rao, G. P. (2005). Stevia: The herbal sugar of the 21st century. *Sugar Technology*, 7(1), 17-24.
- Soejarto, D. D., Kinghorn, A. D., and Farnsworth, N. R. (1982). Potential sweetening agents of plant origin. III. Organoleptic evaluation of *Stevia* leaf herbarium samples for sweetness. *Journal of natural products*, 45(5), 590-599.
- Uçar, E., Turgut, K., Özyiğit, Y., Özek, T. and Özek, G. (2018). The effect of different nitrogen levels on yield and quality of stevia (*Stevia rebaudiana* Bert.). *Journal of Plant Nutrition*, Volume 41 (9).
- Yadav, A.K., S. Singh, D. Dhyani, and P.S. Ahuja. (2011). A review on the improvement of stevia [*Stevia rebaudiana* (Bertoni)]. *Canadian Journal of Plant Science*, 91: 1–27.