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Original Research

Effect of decapitation and exogenous application of gibberellic acid (GA_3) and cytokinin (CK) on some physiological characteristics of stevia

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Abstract: Stevia (*Stevia rebaudiana* B.) has auxiliary buds that often remain dormant for a long time and sometimes remain dormant until the plants change at the reproductive stage. This study was designed out to investigate whether decapitation and exogenous application of plant growth regulators enhance the productivity of stevia through breaking the apical dominance and increasing physiological characteristics. Experiment was carried out as a factorial in randomized complete block design with three replications. Factors were consisted two agricultural practices (Decapitation and No-decapitation) and eight foliar spray including without spray as control, water spray, GA3 (300, 600 and 900 µm) and CK (100, 200 and 400 µm). The results of the present investigation indicated a positive response on number of branches and leaves, leaves and stem fresh weight and total dry weight, in both harvests not only from the decapitation of apical buds but also from foliar application of CK (400 µM). Thus, it can be concluded that the decapitation practices in conjunction with foliar application of CK (400 µM) could be used to increase the dry-leaf yield of stevia. However, further studies are required to standardize the dose of CK (400 µM) to improve the yield and quality of stevia.

Key words: Chlorophyll; Carotenoids; Leaf dry weight; Total dry weight.

Introduction

The *Stevia rebaudiana* Bertoni (2n = 22) is one of 154 members of genus *Stevia* (Asteraceae) of the South American that commonly known as sugar leaf, candy leaf, sweet weed, sweet grass or honey leaf. Stevia is famous for its low calorific value compounds. The active compounds of stevia are steviol glycosides (SGs) including, rebaudioside A, B, C, D, E, F, M, stevioside, steviol bioside, dulcoside A and dulcoside C which are 150-300 times sweeter than sugar and differ to genotype and production environment. Stevioside and rebaudioside A considered being the main sweetening compounds. These compounds are applied for medicinal, food and cosmetic industries (1-8).

It can be a suitable sugar alternative for diabetic patients and has been used by humans without side effects (9). Hence, these plants will achieve to the noticeable place in the natural food market in the near future all around the world (7).

The economic harvest (leaf yield) and content of secondary metabolites depend on the variety, growing situations and agricultural operations. The lateral apices of stevia fixed as auxiliary buds often for a long time, and sometimes permanently until the plant enters their productive stage. Shoot tip removal (decapitation) is an agricultural operation that leads to increasing the number of branches through the release of apical dominance. Since the leaf is the economically important part of stevia yield, it is supposed that decapitation will help obtain higher leaf yield. Apical dominance is the primary causes of repression of auxiliary buds of many agricultural crops. Apical dominance is the control over the outgrowth of auxiliary buds by the shoot tip (10, 11). It is also a type of growth control that is exerted by a biochemical signal from another structure (12). In general, the dominance of the main apex in many plants prevents the development of lateral apices, which remain as auxiliary buds unless the main apex is removed. The yield is the expression of various physiological processes occurring in plants, and these may be altered by agronomic practices (13).

Since the beginning of farming one of the most important goals of human beings has been the control and improvement of plant growth to satisfy human needs. Plant hormones such as abscisic acid, auxins, brassinosteroids (BRs), gibberellins and cytokinins (CKs) are signal molecules present in trace quantities and are actively involved in many biochemical processes (14).

Gibberellic acid (GA_3) has a basic role in many plant growth and development processes (15). Foliar GA_3 application also increased leaf area, leaf fresh weight, leaf dry weight, chlorophyll content, the level of chlorophyll a, b, individual fruit weight, and the number of fertile seeds. All the results suggest that GA_3 application is potentially promising for increasing plant growth and fruit quality of rabbiteye blueberry with delayed ripening time (15).

CK treatment cancels the delay phase in chlorophyll synthesis and increases its rate, prevents chlorophyll breakdown by chlorophyllase, Mg-dechelatase and peroxidase-linked chlorophyll bleaching in higher plants (16). During the present investigation, we have studied the effect of decapitation and exogenous application of gibberelic acid and cytokinin on some biochemical and physiological factors of *stevia rebaudiana* Bertoni.

Table 1. Soil chemical characteristics and classification before cultivation (depth 0-30 cm).

Soil texture	Sand	Silt	Clay	OC	N	pH	EC	P	K	Cu	Zn	Fe	Mn
	(%)						($ds.cm^{-1}$)	(ppm)					
Clay	17.6	35.0	47.4	1.0	0.1	7.6	0.55	5.1	350	1.6	1.4	7.6	6.2

Therefore, the purpose of this study was to approach the best kind and level of plant growth regulators with an agricultural practice (decapitation) in order to reach high biomass.

Materials and Methods

Experimental site

The investigation was carried out in the Experimental Farm at the Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran. The effect of decapitation and foliar spray of gibberelic acid and cytokinin was investigated on some physiological factors of *stevia rebaudiana* Bertoni during the growing seasons of 2015. The experimental farm is in the latitude of 34° 21′N; 47° 9′E and altitude of 1374 meter above sea level. The soil of experimental plots had clay texture. The soil pH was 7.6. Available nitrogen (N), phosphorus (P) and potassium (K) in the soil exist in (Table.1).

Cultural practice

At first, we added 30 ton ha^{-1} rotten manure fertilizer to the soil of the farm and mixed with the soil, and then one-month-old stevia seedlings that had been purchased from the Golsaran-Shomal Cooperative, Guilan Province, Iran, were transplanted in the field on March 31st, 2015 with a spacing of 50 × 45 cm. This crop irrigated at two-day intervals up to one week from the day of transplanting, and at the subsequent stages, the irrigation was given each time that requirements. The weeding operation was done every seven days throughout the life cycle.

Experimental design

This experiment designed with the randomized complete block design (RCBD) with three replications. A Factors including two cultural operations (no decapitation and decapitation) and eight foliar spray treatment (without spray as control, water spray, GA3 (300, 600 and 900 µm) and CK (100, 200 and 400 µm). CK source was 6-BAP (6-benzylaminopurine). Decapitation of the apical buds of primary and secondary branches was done manually on day 45 after transplanting (AT). The prepared solutions of the plant growth regulators were sprayed 1000 L ha^{-1}. The foliar spray PGRs solutions were performed on day 47 AT.

Sampling and analysis of plant

For measurement of growth factors selected three plants randomly and cut at ground level from each plot at day 85 AT. Sampling for biochemical measurements were done one day before each harvesting. The shoots were harvested with some secateurs and removed from 15 cm above soil level. The fresh weight of above ground (leaf and stem) recorded after washing with tap water. The moisture remained on the surface of plant absorbed by using blotting paper. Then leaf and stem fresh weights were measured. After that, the samples were dried in oven at 70 ± 2°C for 48 hours. Plant height and number of branches per plant were also recorded at the time of harvest. For chlorophyll (Chl. a, b and Total) and carotenoid determination, stevia leaves were collected from the middle portion of the plants from each plot. Initially, major veins were discarded from collected samples, and 200 mg leaf sample was separated from each leaf for extracting the Chlorophyll. Chlorophyll was extracted in a solution of 80% acetone (v/v) following a method of Arnon (1949) (18). Finally, the absorption of the extracts was recorded at 645 and 663 nm. Chlorophyll a, b, total and carotenoids were calculated to mg g^{-1} leaf from the absorption values as standard equations suggested by Arnon (1949).

Statistical analysis

Data were subjected to the analysis of variance used SAS software. The data on different parameters were evaluated using ANOVA and means of traits were compared based on the least significant difference test (LSD) at the 0.05 probability level.

Results

Photosynthetic pigments

The effect of decapitation on chlorophyll a, b, total and carotenoid content in leaves of stevia was not significant. However, the foliar application of different plant growth regulators significantly influenced chlorophyll a, b, total and carotenoid content in leaves of stevia in both harvests (Table 2). Among the plant growth regula-

Table 2. Analysis of variance for the photosynthetic pigments of stevia treated with decapitation and different plant growth regulators (GA_3 and CK) in tow harvest.

Source of variance	df	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoid	
		Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Block	2	0.009 ^{ns}	2.980 ^{ns}	0.0167 ^{ns}	0.0756 ^{ns}	0.0460 ^{ns}	2.112 ^{ns}	0.0649 ^{ns}	0.003 ^{ns}
Decapitation (D)	1	0.117 ^{ns}	0.195 ^{ns}	0.001 ^{ns}	0.0085 ^{ns}	0.144 ^{ns}	0.122 ^{ns}	0.0117 ^{ns}	0.065 ^{ns}
Plant growth regulators (P)	7	16.09 **	36.79**	1.964 **	122.56**	28.60 **	56.80**	1.321 **	2.130**
Interaction D×P	7	0.293 ^{ns}	0.098 ^{ns}	0.0315 ^{ns}	0.0254 ^{ns}	0.393 ^{ns}	0.1134 ^{ns}	0.020 ^{ns}	0.0418 ^{ns}
Error	30	0.2371	1.1253	0.0365	0.0241	0.290	1.226	0.0229	0.0289
CV (%)	-	6.62	12.86	8. 93	7.48	5.67	10.73	7.70	8.56

*, ** represent significance at the 0.05 and 0.01 probability level, respectively and ns denotes non-significance.

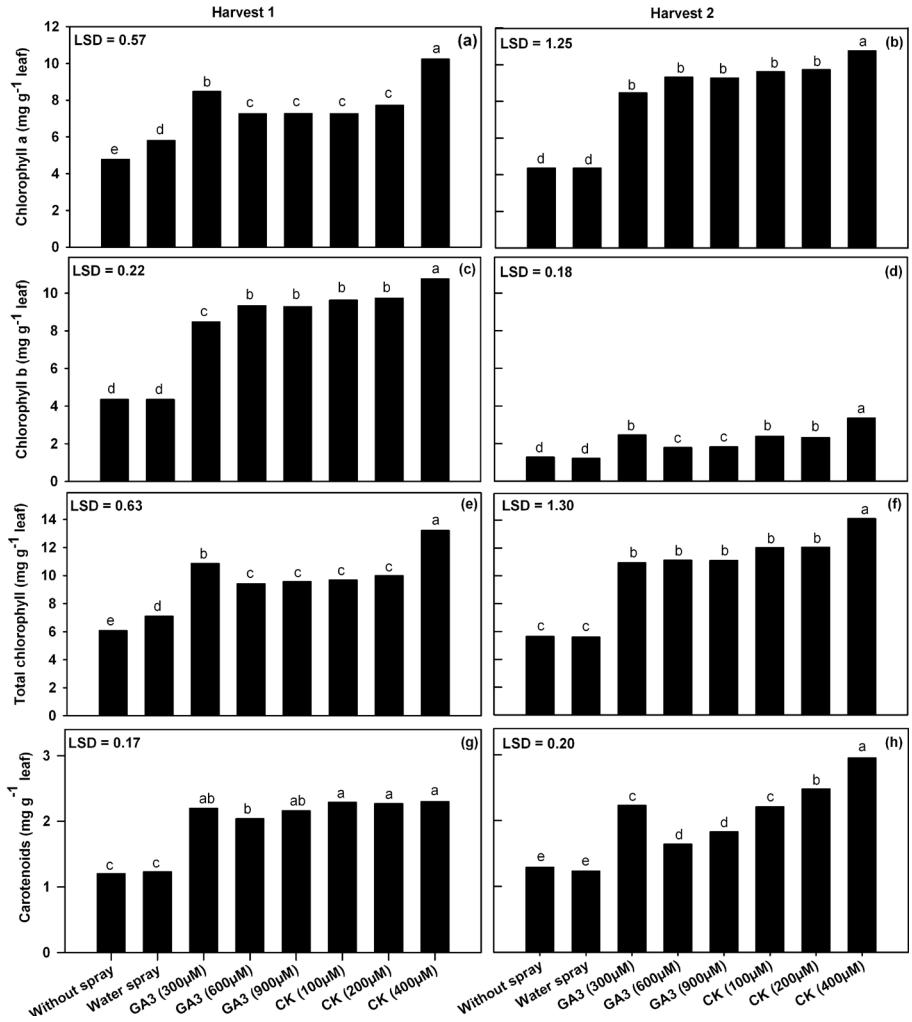

Figure 1. Effect of different plant growth regulators (GA_3 and CK) on chlorophyll a (a and b), chlorophyll b (c and d), total chlorophyll (e and d) and carotenoids (g and h) of stevia in two harvests.

tors treatments, application of CK (400 µM) resulted in significantly higher chlorophyll a, b, total (mg g^{-1} leaf) in stevia leaves compared to other treatments in both harvest (Fig. 1). The most contents of carotenoid (mg g^{-1} leaf) in the first harvest observed in CK (400, 200 and 100 µM) that in these treatments there were no significant differences (Fig. 1). Carotenoid content has the most amounts in the second harvest under the influence of CK (400 µM) application (Fig. 1).

Morpho-physiological traits

There was no significant effect of decapitation on plant height in the first and second harvest (Table 3). Nevertheless, the foliar application of different plant growth regulators significantly influenced plant height in both harvests (Table 3). Decapitation and foliar application of different plant growth regulators significantly affected morpho-physiological parameters such as the number of branches and leaves, leaves and stem fresh weight in both harvests and also leaf dry weight in the first harvest (Table 3). Furthermore, interaction between decapitation and foliar application of different plant growth regulators, on mentioned traits was significant (Table 3). Effect of the foliar application of different plant growth regulators on leaf dry weight in the plant in the second harvest was significant (Table 3). Stem dry weight in the plant in the first harvest was influenced by decapitation and foliar application of different plant growth regulators. Decapitation, foliar application of

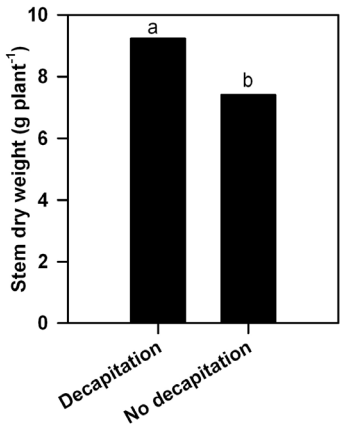

Figure 2. Effect of decapitation on stem dry weight in the first harvest of stevia.

different plant growth regulators and their interaction affected stem dry weight in the plant in the second harvest (Table 3). In addition, this two factors and their interaction significantly affected on total dry weight in the plant in both harvests (Table 3).

The effect of decapitation on stem dry weight in the plant of stevia (9.24 g) was significantly greater than no decapitation (7.41). This result has been shown in Fig. 2. The highest plant height in the first harvest (62.88 cm) and the second harvest (49.88 cm) obtained in GA_3 (900 µM). This result has been brought in Fig.5. Maximum leaf dry weight in the second harvest and maximum stem dry weight in the first harvest obtained under

Table 3. Analysis of variance for the morpho-physiological characteristics of stevia treated with decapitation and different plant growth regulators (GA_3 and CK).

Source of variance	df	Plant height		Branch number		Leaf number		Leaf fresh weight	
		Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Block	2	5.43^{ns}	5.43^{ns}	0.062^{ns}	1.56^{ns}	16.18^{ns}	16.18^{ns}	1.27^{ns}	1.27^{ns}
Decapitation (D)	1	9.63^{ns}	9.63^{ns}	229.6 **	5742.1**	26180.0**	26180.0**	1189.0 **	1189.0**
Plant growth regulators (P)	7	774.3**	774.3**	245.1**	6129.6**	45578.4**	45578.4**	1799.1 **	1799.1**
Interaction D×P	7	7.16 ^{ns}	7.16^{ns}	9.44 **	236.2**	347.5*	347.5**	24.16**	24.16**
Error	30	6.93	6.93	1.01	25.45	135.00	135.00	5.04	5.04
CV (%)	-	5.77	8.90	5.62	5.62	4.06	4.09	5.18	5.30
Source of variance	**df**	**Stem fresh weight**		**Leaf dry weight**		**Stem dry weight**		**Total dry weight**	
		Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Block	2	0.03 ^{ns}	0.13 ^{ns}	0.24 ^{ns}	0.14 ^{ns}	0.28 ^{ns}	0.005 ^{ns}	0.82 ^{ns}	0.14 ^{ns}
Decapitation (D)	1	750.1 **	3003.2 **	70.47 **	132.2 ^{ns}	40.18 **	120.1 **	217.0 **	504.4 **
Plant growth regulators (P)	7	1277.7 **	5111.1 **	114.5 **	199.9 **	58.66 **	204.4 **	336.7 **	808.5 **
Interaction D×P	7	14.71 **	58.86 **	1.08 **	2.69 ^{ns}	0.61 ^{ns}	2.35 **	2.08 **	9.78 **
Error	30	2.69	10.76	0. 22	0.55	0.29	0.43	0.57	1.12
CV (%)	-	4.12	4.12	4.03	6.45	6.49	4.12	3.77	3.86

*, ** represent significance at the 0.05 and 0.01 probability level, respectively and ns denotes non-significance.

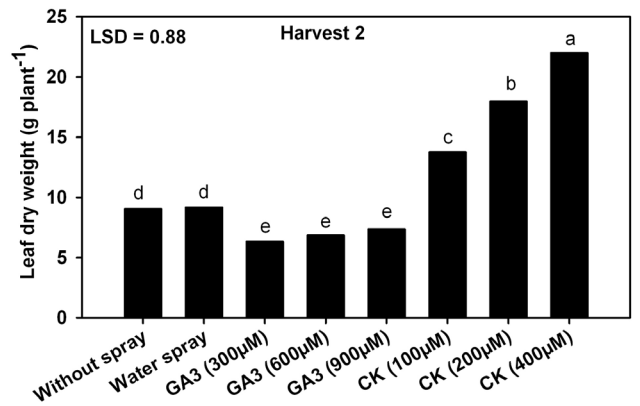

Figure 3. Effect of different plant growth regulators (GA_3 and CK) on leaf dry weight in the second harvest of stevia.

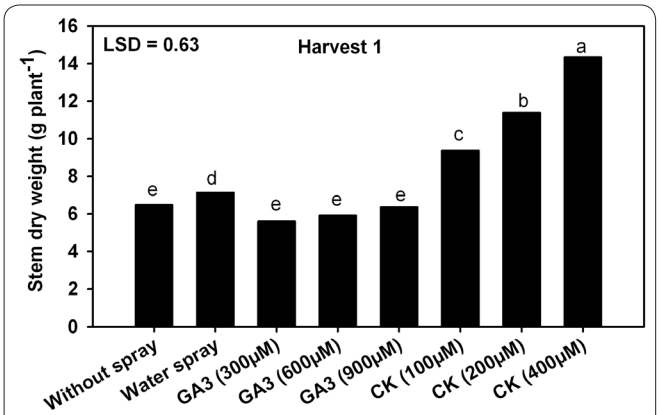

Figure 4. Effect of different plant growth regulators (GA_3 and CK) on stem dry weight in the first harvest of stevia.

the impact of application CK (400 µM). These results have been shown in Fig.3 and 4.

Treatment combination of decapitation and foliar spray of CK (400 µM) made it possible to achieve the highest number of branches, number of leaves, leaves fresh weight, stem fresh weight and total dry weight in plant in both harvests (Table 4 and Fig. 6) and leaves dry weight in plant in the first harvest and stem dry weight in plant in the second harvest (Table 4).

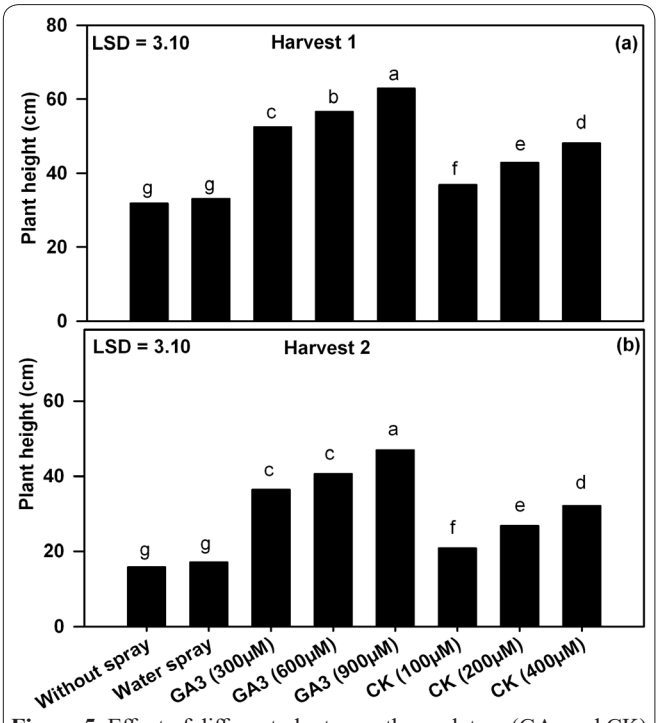

Figure 5. Effect of different plant growth regulators (GA_3 and CK) on plant height in two harvests (a and b) of stevia.

Discussion

The foliar application of different plant growth regulators significantly influenced chlorophyll a, b, total and carotenoid content in leaves of stevia in both harvests (Table 2). Among the plant growth regulators, application of CK (400 µM) resulted in significantly higher chlorophyll a, chlorophyll b, total chlorophyll (mg g^{-1}leaf) content in stevia leaves compared to the other treatments in both harvests (Fig. 1). Plant hormones such as abscisic acid, auxins, brassinosteroids (BRs), gibberellins and cytokinins (CKs) are signal molecules present in trace quantities and are actively involved in many biochemical processes (15). Photosynthesis enhancement is an often observed feature in plant cells in

Table 4. The effects of decapitation and different plant growth regulators (GA_3 and CK) on morpho-physiological characteristics of stevia.

Treatments		Leaf fresh weight (g plant^{-1})		Stem fresh weight (g plant^{-1})		Leaf dry weight (g plant^{-1})	Stem dry weight (g plant^{-1})
		Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Decapitation							
	Without spray	41.00 ^{g}	40.00^{g}	37.60 ^{g}	75.20^{g}	10.85 ^{g}	15.04^{g}
	Water spray	43.00 ^{g}	42.00^{g}	39.26 ^{g}	78.53^{g}	11.43 ^{g}	15.70^{g}
	GA3 (300µM)	34.66 ^{h}	33.66^{h}	32.26 ^{h}	64.53^{h}	9.80 ^{h}	12.90^{h}
	GA3 (600µM)	36.66 ^{h}	35.66^{h}	33.03 ^{h}	66.06^{h}	9.75 ^{h}	13.21^{h}
	GA3 (900µM)	35.66 ^{h}	34.66^{h}	33.90 ^{h}	67.80^{h}	9.87 ^{h}	13.56^{h}
	CK (100µM)	52.66 ^{e}	51.66^{e}	47.58 ^{e}	95.16^{e}	14.30 ^{e}	19.03^{e}
	CK (200µM)	65.46 ^{c}	64.46^{c}	58.03 ^{c}	116.06^{c}	17.31 ^{c}	23.21^{c}
	CK (400µM)	77.23 ^{a}	76.23^{a}	68.40 ^{a}	136.80^{a}	20.30 ^{a}	27.36^{a}
No Decapitation							
	Without spray	30.60 ^{i}	29.60^{i}	27.76 ^{ij}	55.53^{ij}	7.58 ^{j}	11.10^{ij}
	Water spray	29.30 ^{ij}	28.30^{ij}	28.56 ^{i}	57.13^{i}	8.88 ^{i}	11.42^{i}
	GA3 (300µM)	20.63 ^{k}	19.63^{k}	20.60 ^{l}	41.20^{l}	6.18 ^{k}	8.24^{l}
	GA3 (600µM)	21.76 ^{k}	20.76^{k}	23.36 ^{k}	46.73^{k}	6.86 ^{jk}	9.34^{k}
	GA3 (900µM)	25.83 ^{j}	24.83^{j}	25.33^{jk}	50.66^{jk}	7.32 ^{j}	10.13^{jk}
	CK (100µM)	47.16 ^{f}	46.16^{f}	43.50 ^{f}	87.00^{f}	12.51 ^{f}	17.40^{f}
	CK (200µM)	59.46 ^{d}	58.46^{d}	53.56 ^{d}	107.13^{d}	15.83 ^{d}	21.42^{d}
	CK (400µM)	71.96 ^{b}	70.96^{b}	64.10 ^{b}	128.20^{b}	19.05 ^{b}	25.64^{b}
LSD		3.74	3.74	2.73	5.47	0.78	1.09

Means with the same letter are not significantly different according to LSD (0.05).

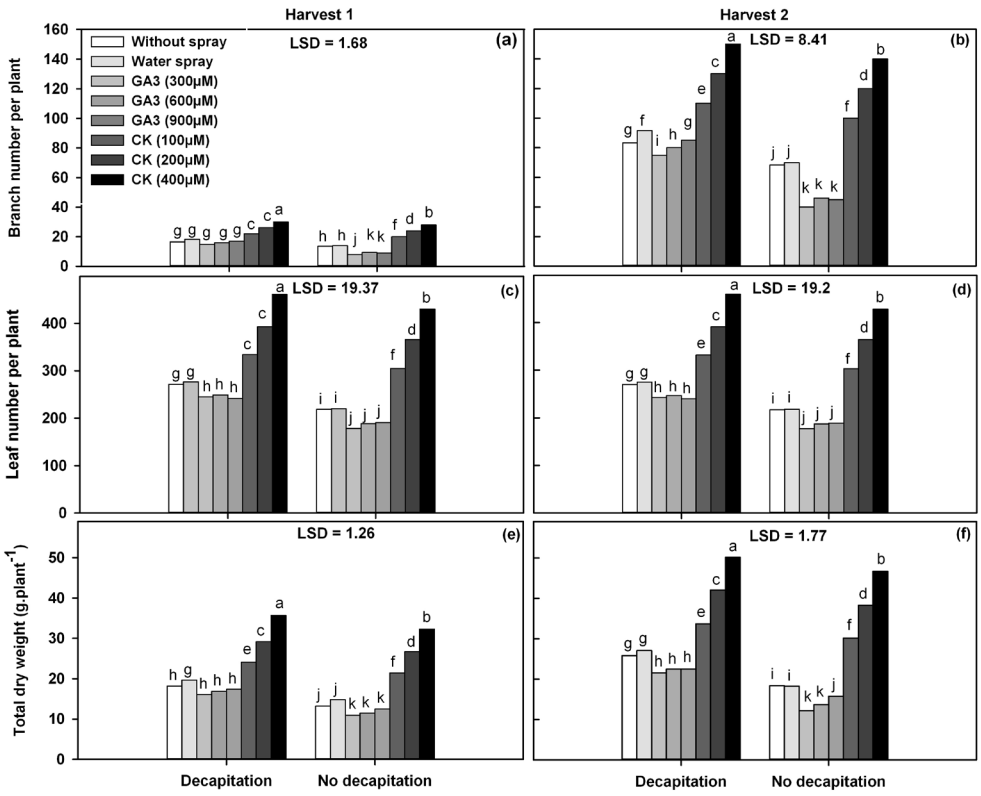

Figure 6. The effect of decapitation and different plant growth regulators (GA_3 and CK) on branch number per plant (a and b), leaf number per plant (c and d) and total dry weight (e and f) in tow harvest.

response to BRs and CKs (18-20). Cytokinins cause a delay in leaf aging. However, the application of cytokinin does not completely prevent aging, but it is extremely effective, especially when directly spray on the plant (21).

Bajguz and Piotrowska-Niczyporuk (2014) reported the increase in the total content of chlorophylls in algal cells exposed to CKs without BRs and BRs without CKs was observed in 48 hours of algal cultivation (22). On the other hand, C. vulgaris possessed much higher content of total chlorophylls in response to the BRs combined with CKs. High accumulation of chlorophyll level and increase in net photosynthesis rate was also observed in leaves of V. radiate sprayed with Kin and

28-homoBL (18).

The effect of decapitation on stem dry weight of stevia (9.24 g) was significantly greater than no decapitation (7.41); this result is in Fig. 2. The highest plant height in the first harvest (62.88 cm) and the second harvest (49.88 cm) obtained in GA3 (900 µM), this result is in Fig. 5. The prolongation of the cells is the most noticeable effect of gibberellic acid on the growth of plants that usually increases the growth of plants happens by extending the internodes of the stems (15). Mostafa et al. (2005) said that from gibberellic acid hormone can be used to increase the growth characteristics and amount of essential oil of aromatic plants (25). Abd El- Aal et al. (2008) reminded that Gibberellins increase cell division and prolongation, so the exogenous application of gibberellic acid can increase Branch growth, photosynthesis and dry matter accumulation (15). Santos et al. (1998) stated that with the use of gibberellic acid, the growth of carrot leaves increased. However, about stevia in this study, we didn't catch the same result. Probably in the different plant the conditions are various (24). Emongor (2007) reported that gibberellic acid application on black-eye pea plant about seven days after planting caused significant increases in vegetative growth (25). The results of the study by Akter et al. (2007) showed that different concentrations of gibberellic acid had a significant effect on plant height (26). These results agree with findings in this study. Gibberelli cacid, as a plant growth regulator, increase the cell division and development of apical and lateral buds. However, gibberellic acid by this way increases the absorption of nutrient (27).

The gibberellic acid application also leads to prolongation of the stem internodes that it causes increasing fresh and dry weight of up ground plant part (4). This result also confirms the increasing of height plant by using GA3 in this study. It seems like that gibberellic acid has an increasing effect on plant growth and stem height and increase them. So in this way gibberellic acid increased number and length of cells in the stem. Finally, it leads to increasing fresh and dry weight of the stem (25).

Maximum dry leaf weight in the second harvest and maximum dry stem weight in the first harvest obtained under the impact of application CK (400 µM), this result is in Fig. 3 and Fig. 4. Treatment combination of decapitation and foliar spray of CK (400 µM) made it possible to achieve the highest number of branches, number of leaves, total dry weight, leaves fresh weight and stem fresh weight in plant, in both harvests (Fig. 6, Table 4) and leaves dry weight in plant in the first harvest and stem dry weight in plant in the second harvest (Table 4). Treatment combination of decapitation and foliar spray of CK (400 µM) made it possible to achieve the highest number of branches, the number of leaves, total dry weight, leaves fresh weight, stem fresh weight in the plant, in both harvests (Fig. 6 and Table 4).

Cytokinins affect on many physiological and developmental processes such as nutrient transportation, formation and activation of the apical meristem, flower development, breaking of lateral buds dormancy and germination of seeds. However, cytokinins regulate many cellular processes, the main task and the main factor in identifying this group of plant hormones is cell division control. In addition to the role in cell division of cytokinin, it has many other effects such as vascular development, apical dominance and aging leaves (13). Cytokinins accelerate the development of chloroplasts. If the pale leaves treated with cytokinin before lightening, extensive granular chloroplasts form and Photosynthetic enzymes are produced more rapidly until lightening time.

The results of the present investigation indicate a positive response in the number of branch and leaf, leaf and stem fresh weight as well as the total dry weight of plant, in both harvests not only from the decapitation of apical buds but also from foliar application of CK (400 µM). Thus, it can be concluded that the decapitation practices in conjunction with foliar application of CK (400 µM) may be used to increase the dry leaf yield of stevia. However, further studies are required to standardize the dose of CK (400 µM) needed to improve the yield and quality of stevia.

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