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Effect of NPK Fertilization on the Growth, Yield, Quality and Mineral Nutrition of New Sweet Plant in Morocco (*Stevia rebaudiana* Bertoni)

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Abstract

Stevia (*Stevia rebaudiana* Bertoni) is an herbaceous perennial plant of the *Asteraceae* family, originating from the Amambay region in the north-east of Paraguay, where it grows wild in sandy soils. Dry leaves are the economic part of the stevia plant, with a high concentration of steviol glycosides, which are many times sweeter than sugarcane and sugarbeet but importantly without any calories. Fertilizer requirement for stevia is moderate and varies according to the environment and soil type. Due to the short time of stevia introduction as a new crop in Morocco, there is no information available on nutrient requirement. The aim of the present work is to determine the optimum nitrogen (N), phosphorus (P), and potassium (K) levels for higher dry leaf yield and steviol glycosides content and their accumulation in stevia in north-western Moroccan conditions. The experiment consisted of 27 fertilization treatments combinations of N (100, 200, and 300 kg ha^{-1}), P (50, 100, and 150 kg ha^{-1}), and K (80, 160, and 240 kg ha^{-1}) and a control treatment, each in three replicates. The results indicated that significantly higher fresh biomass yield, fresh and dry leaf yield, and total steviol glycosides yield were obtained with T24 treatment (300N, 100P, 240K) (96.53, 69.87, 19.56, and 2.13 g $plant^{-1}$, respectively). Also, T24 led to higher N content (1.81%) than the control (0.40%). However, higher P and K contents were obtained with T25 (300N, 150P, 80K) and T3 (100N, 50P, 240K) treatments, respectively. The growth parameters viz., plant height and stem diameter were significantly higher with T16 treatment (200N, 150P, 80K) while, the stevioside and total steviol glycoside contents were higher in T6 (100N, 100P, 240K) stevia leaves. The T24 could be considered as an economically optimum level of nutrients for stevia.

Keywords

Stevia, Steviol Glycosides, Nitrogen, Phosphorus, Potassium, Dry Leaf, Yield

1. Introduction

Stevia (*Stevia rebaudiana* Bertoni) is an herbaceous perennial plant of the *Asteraceae* family, originating from the Amambay region in the north-east of Paraguay, where it grows wild in sandy soils near streams on the edges of marshland, acid infertile sand or muck soils [24]. Dry leaves are the economic part of the stevia plant [37], with a high concentration of steviol glycosides (SG), possible substitutes of synthetic sweeteners [39] which are many times sweeter than sugarcane and sugarbeet but importantly without any calories [8]. The main SG in stevia leaf are stevioside (STV) (5–10% of dry leaf weight), which is about 300 times sweeter than sucrose [11] and rebaudioside A (Reb A) (2–4%), which is more suited than STV for use in foods and beverages due to its pleasant taste [44]. Commercial exploitation of stevia started in 1970 in Japan [36] and then extended to China, Brazil, Paraguay, Mexico, Russia, Indonesia, Korea, USA, India, Tanzania, Canada and Argentina [32]. European regulatory bodies including the joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) have now agreed that SG is safe for all populations to consume and is a suitable sweetening option for diabetics. Effective from December 2nd, 2011, the EU has approved it use as a food additive [12]. Stevia is relatively unknown in Morocco, where it can be a new sweet crop [1].

The amount of SG depends on total biomass yield, which further depends on the climate and agro-techniques [19], [20]. Among the agro-techniques, reliable nutrient supply is the most important factor for higher crop yield. Among the 17 essential plant nutrients, N, P and K are the most often limiting macronutrients for plant growth and development. Nitrogen is an essential element of key macro-molecules such as proteins, nucleic acids, some lipids, and chlorophylls [34]. Phosphorus is also a component of nucleic acids, phospholipids, and ATP [41]. Potassium, third most essential macronutrient of plant, plays a central role in many fundamental metabolic processes, such as turgor driven movements, osmoregulation, control of membrane polarization and protein biosynthesis [10]. Thus, plants cannot perform properly without a reliable supply of these nutrients. Moreover, high dose fertilizer mainly N is harmful for soil health, especially when applied above the economic optimum dose.

Nutritional requirements for stevia are low to moderate [14] since this crop is adapted to poor quality soils in its natural habitat at Paraguay. While, [15] reported that nutritional dose varies according to the environment and soil type. Under average climatic conditions and soil type 70 kg Nitrogen, 35 kg Phosphorus and 45 kg potassium per hectare is recommended [43]. [32] have earlier studied the interactive effects of crop ecology and plant nutrition on yield and secondary metabolites of stevia in northern India. [3], have studied the effect of nitrogen, phosphorus and potassium levels on growth and yield of stevia in medium black, clayey soil under south of India. It was shown that the application of foliar nutrients led to an increase in chlorophyll, nitrogen, and potassium content in leaves but not in SG content [33]. Due to the short time of stevia introduction as a new crop in Morocco, there is no information available on nutrient requirement. The aim of the present work is to determine the optimum nitrogen (N), phosphorus (P), and potassium (K) levels for higher dry leaf yield and SG content and their accumulation in stevia in north-western Moroccan conditions.

2. Materials and Methods

2.1. Study Location

The study was carried out during stevia growing period from 25th March to 17th August, 2014 in the Regional Centre of Agronomic Research of Rabat in Morocco (INRA) (34.21 N, 6.40 E, 10.5 m above mean sea-level). The location (Rabat) represents the sub humid region of north-western Morocco, with mean maximum temperature of 27.1°C in August and mean minimum of 8°C in January**.** The average annual rainfall received is about 554 mm, of which about 74 percent is received during November to March. During the crop growth period daily maximum temperature ranged from 26.3 to 28.2°C, the minimum temperature ranged from 14.8 to 18.3°C and the mean relative humidity ranged between 66.8–86.6%. Total rainfall received during the crop growth season was 4.6 mm. These climatic data were measured at a height of 2 m by an automatic weather station (iMETOS, Pessl Instruments, Austria), located near the experimental site.

2.2. Experimental Design and Treatments

The selected seed for that experiment belongs to the INRA variety. The sowing was performed into plug trays filled with land and commercial substrate on March 25th, 2014 and watered to field capacity (FC) by tap water in the greenhouse. Two-month-old the uniform seedlings were transplanted in the plastic pots on May 27th, 2014, with two plants per pot. The 10 L pots were filled with 1 kg of gravel at the bottom for drainage and 6 kg of sandy soil. Before application of mineral fertilizers. The soil was analysed in the laboratory of Research Unit on Environment and Conservation of Natural Resources INRA, RCAR of Rabat. The soil contained 5.1% clay, 11.7% silt, and 80.8% sand. The organic matter content was 2.5%, the pH was 8.15 and the N, P, and K contents were 39.7, 7.6, and 20.3 ppm, respectively. Soil moisture at field capacity was 13.44% and soil moisture at permanent wilting point was 4.71%. Soil density (ρ) was 1.4 g cm^{-3}, which used to convert doses of NPK from kg ha^{-1} to g pot^{-1}. All pots were placed in open field and irrigated near the field capacity since this experiment was conducted during stevia growing season. The experiment consisted of 27 fertilization treatments combinations comprising three levels of N (100, 200, and 300 kg ha^{-1}), three levels of P (50, 100, and 150 kg ha^{-1}), and three levels of K (80, 160, and 240 kg ha^{-1}) and a control treatment without any nutrients, each in three replicates totalling 84 experimental pots arranged according to a randomized complete block design. Details of treatments are shown in the tables 1, 2 and 3. The NPK fertilizers were applied in the form of ammonium nitrate (33% N), triple superphosphate (45% phosphorus pentoxide (P_2O_5)), and muriate of potash (50% potassium oxide (K_2O)), respectively. A half dose of N and full dose of P and K as per treatment were applied at the time of transplanting, while remaining half dose of N was applied at 45 days after transplanting. The plants of the whole pots were harvested manually 10 cm above the base of the stem [27] at 85 days after transplanting on August 17th, 2014, when the concentration of steviol glycoside is maximum [7]. Leaves and stems were separated and used for further data analysis.

2.3. Growth and Yield Analysis

Plant height and stem diameter of stevia plants were recorded at harvest. The plant height was measured with a meter ruler from ground to the base of the fully opened leaf and the stem diameter was measured with slide calipers up to 0.01 mm accuracy. Biomass yield (total fresh leaf and stem yield), fresh leaf yield, and dry leaf yield were determined in each plant. We estimated the fresh biomass, fresh and dry leaf yield per plant using one digital scale with precision of 0.01 g. Leaves were dried at 50°C temperature in hot air dryer for 6 hours and stored in clean gunny bags. At this temperature, the quality of dried leaves produced, in terms of colour, sweetness and nutrient content, was better compared with drying at 70°C [40]. Dry leaf had an important role in stevia extract in term of quality [48].

2.4. Determination of NPK in Leaf

After recording growth and yield data, the dried stevia leaf samples were prepared with a laboratory grinder having a sieve spacing of 2 mm to determine nitrogen, phosphorus, and potassium content in the leaf. Total nitrogen content was determined by using the Macro Kjeldahl digestion and distillation method [35], while total phosphorus and potassium were determined using a colorimetric method [31] and flame photometer (model CL378) [46], respectively.

2.5. Steviol Glycosides Analysis

For determination of steviol glycosides for all plants, dry leaves of stevia obtained during this experiment were ground in a laboratory grinding mill to produce powder particles of 0.10 mm in size, and were kept at ambient temperature until they were used for the analysis to assess the contents of stevioside (STV), rebaudioside A (Reb A) and total steviol glycosides (STV; Reb, A, B, C, D and F; steviolbioside; rubudioside and dulcoside A) as influenced by NPK fertilizers. STV (%), Reb A (%) and total SG (%) were determined in the powdered stevia leaves sent to the STEVIA NATURA Company of France. The SG yield was estimated by multiplying dry leaves yield by the content of SG in leaves.

2.6. Statistical Analysis

Data obtained were analyzed by the analysis of variance (ANOVA) using Statistical Analysis System ver. 9.1 (SAS Institute Inc., Cary, NC., USA), and means were compared using Duncan's multiple range test (DMRT) at the 0.05 significance level.

3. Results

3.1. Growth and Yield Parameters

The mean data on plant height, stem diameter, fresh biomass yield, fresh leaf yield, and dry leaf yield are presented in table 1. These parameters were significantly influenced by the interaction effects of different levels of nitrogen (N), phosphorus (P) and potassium (K) compared to the control. Treatment T16 (200:150:80 kg ha^{-1} NPK) remained statistically at par with T5 (100:100:160 kg ha^{-1} NPK) but recorded significantly higher plant height (71 cm) than remaining treatments and absolute control (30 cm). T16 and T5 recorded 57.75 percent and 56.93 percent higher plant height as compared to the control, respectively. Stem diameter data also followed the same trend as plant height. Highest stem diameter (9.59 mm) was possible with T16 which was on par with T4 (100:100:80 kg ha^{-1} NPK) and both were significantly higher as compared to all other treatments and control (4.88 mm). T16 recorded 49.11 percent higher stem diameter as compared to the control. Treatment T24 (300:100:240 kg ha^{-1} NPK) produced significantly greater fresh biomass yield at harvest (96.53 g plant^{-1}) as compared to remaining treatments and control (17.70 g plant^{-1}). Likewise, fresh leaf yield and dry leaf yield were also significantly greater in the T24 treatment (69.87 and 19.56 g plant^{-1}, respectively) as compared to all other treatments and the control (11.43 and 3.33 g plant^{-1}, respectively). The control significantly decreased fresh biomass yield, fresh leaf yield, and dry leaf yield until 81.66%, 83.64%, and 82.97%, respectively, compared to T24.

Table 1. *Effect of NPK fertilization on growth and yield parameters of stevia.*

Treatment	Parameters				
	Plant height (cm)	Stem diameter (mm)	Fresh biomass (g plant^{-1})	Fresh leaf (g plant^{-1})	Dry leaf (g plant^{-1})
T0	30.00o	4.88o	17.70m	11.43m	3.33o
T1	33.67n	5.49n	27.68k	17.62kl	5.07mn
T2	50.00l	6.29lm	22.42l	13.51m	3.35o
T3	48.33l	7.41efghi	25.56kl	13.65m	4.26no
T4	48.33l	9.59a	35.16ij	18.66kl	6.91k
T5	69.67a	8.45bc	42.37h	20.77k	6.33kl
T6	48.67l	6.75kl	29.12k	17.37l	5.15mn
T7	48.67l	5.86mn	27.52k	19.74kl	5.91lm
T8	58.33fgh	7.98cde	37.54i	26.57j	6.99k
T9	59.67def	7.34fghij	84.58b	48.73d	12.56def
T10	62.67bc	8.35bc	62.33ef	41.81f	10.90h
T11	50.67kl	7.20hij	53.69g	35.33h	11.65fgh
T12	51.00kl	7.50efgh	52.53g	39.52fg	13.88c
T13	53.33jk	7.70defg	59.92f	42.27f	12.74de
T14	45.33m	6.98ijk	33.69j	28.03ij	8.69j

Treatment	Parameters				
	Plant height (cm)	Stem diameter (mm)	Fresh biomass (g plant^{-1})	Fresh leaf (g plant^{-1})	Dry leaf (g plant^{-1})
T15	51.33kl	8.37bc	53.06g	39.21fg	11.53fgh
T16	71.00a	9.59a	51.10g	27.72ij	9.01ij
T17	64.33b	8.20cd	52.79g	35.37h	9.77i
T18	62.00bcd	7.97cdef	63.27def	39.60fg	12.54def
T19	55.67hij	7.26hij	77.35c	53.98bc	13.23cd
T20	63.00bc	7.94cdef	60.49f	35.74h	12.36defg
T21	59.00efg	8.83b	54.62g	37.68gh	11.98efg
T22	56.33ghi	8.84b	84.45b	55.76b	16.99b
T23	60.33cdef	6.84jkl	37.27ij	30.18i	14.16c
T24	54.33ij	7.30ghij	96.53a	69.87a	19.56a
T25	58.33fgh	7.65defg	42.14h	27.60ij	8.77j
T26	61.33cde	8.05cde	65.27de	45.67e	11.45gh
T27	48.33l	7.71defg	66.54d	51.14cd	13.17cd

* Means followed by different letters in each column are significantly different (Duncan multiple range test at the 5% significance level).
T0 (Control), T1 (100:50:80 kg ha^{-1} NPK), T2 (100:50:160 kg ha^{-1} NPK), T3 (100:50:240 kg ha^{-1} NPK), T4 (100:100:80 kg ha^{-1} NPK), T5 (100:100:160 kg ha^{-1} NPK), T6 (100:100:240 kg ha^{-1} NPK), T7 (100:150:80 kg ha^{-1} NPK), T8 (100:150:160 kg ha^{-1} NPK), T9 (100:150:240 kg ha^{-1} NPK), T10 (200:50:80 kg ha^{-1} NPK), T11 (200:50:160 kg ha^{-1} NPK), T12 (200:50:240 kg ha^{-1} NPK), T13 (200:100:80 kg ha^{-1} NPK), T14 (200:100:160 kg ha^{-1} NPK), T15 (200:100:240 kg ha^{-1} NPK), T16 (200:150:80 kg ha^{-1} NPK), T17 (200:150:160 kg ha^{-1} NPK), T18 (200:150:240 kg ha^{-1} NPK), T19 (300:50:80 kg ha^{-1} NPK), T20 (300:50:160 kg ha^{-1} NPK), T21 (300:50:240 kg ha^{-1} NPK), T22 (300:100:80 kg ha^{-1} NPK), T23 (300:100:160 kg ha^{-1} NPK), T24 (300:100:240 kg ha^{-1} NPK), T25 (300:150:80 kg ha^{-1} NPK), T26 (300:150:160 kg ha^{-1} NPK), T27 (300:150:240 kg ha^{-1} NPK).

3.2. Nutrient (NPK) Contents in Leaf

The effects of different combinations of NPK fertilization on nitrogen, phosphorus, and potassium contents in dry leaf of stevia are presented in table 2. All of the above parameters were significantly influenced by different NPK combinations compared to the control. Significantly higher nitrogen content (1.81%) in dry leaf was recorded with 300:100:240 kg ha^{-1} NPK (T24) as compared to all other treatments and absolute control. The lowest nitrogen content was with the control (0.40%). However, application of 300:150:80 kg ha^{-1} NPK (T25) recorded significantly higher phosphorus content (1.18%) in dry leaf as compared to the control (0.08) and other treatments. The combination of 100:50:240 kg ha^{-1} NPK (T3) recorded significantly higher potassium content (2.41%) in dry leaf as compared to the control (0.82) and other treatments but remained statistically at par with T6 (100:100:240 kg ha^{-1} NPK) and T18 (200:150:240 kg ha^{-1} NPK). The potassium content was decreased in control stevia dry leaf than T3, T6, and T18 (65.98%, 65.55%, and 65.40%, respectively).

Table 2. *Effect of NPK fertilization on NPK (%) content in dry leaf of stevia.*

Treatment	Parameters		
	Nitrogen (%)	Phosphorus (%)	Potassium (%)
T0	0.40m	0.08k	0.82m
T1	0.86j	0.11i	1.21j
T2	0.91i	0.10j	1.80gh
T3	0.82k	0.12hi	2.41a
T4	0.79l	0.18fg	1.17k
T5	0.82k	0.16g	1.77h
T6	0.86j	0.17fg	2.38ab
T7	0.83k	0.22e	1.16k
T8	0.91i	0.24bcd	1.90f
T9	0.93i	0.23de	2.29de
T10	1.39e	0.10j	1.17k
T11	1.27g	0.11i	1.83g
T12	1.33f	0.12hi	2.36bc
T13	1.41e	0.19f	1.13kl
T14	1.25h	0.26b	1.78h
T15	1.28g	0.18fg	2.28e
T16	1.40e	0.25bc	1.15kl
T17	1.38e	0.24bcd	1.90f
T18	1.31f	0.23de	2.37abc
T19	1.68d	0.13h	1.13kl
T20	1.70cd	0.12hi	1.81gh
T21	1.72c	0.11i	2.30de
T22	1.69d	0.17fg	1.11l
T23	1.75b	0.17fg	1.70i
T24	1.81a	0.17fg	2.27e
T25	1.77b	1.18a	1.12kl
T26	1.69d	0.25bc	1.80gh
T27	1.75b	0.25bc	2.33cd

* Means followed by different letters in each column are significantly different (Duncan multiple range test at the 5% significance level).
T0 (Control), T1 (100:50:80 kg ha^{-1} NPK), T2 (100:50:160 kg ha^{-1} NPK), T3 (100:50:240 kg ha^{-1} NPK), T4 (100:100:80 kg ha^{-1} NPK), T5 (100:100:160 kg ha^{-1} NPK), T6 (100:100:240 kg ha^{-1} NPK), T7 (100:150:80 kg ha^{-1} NPK), T8 (100:150:160 kg ha^{-1} NPK), T9 (100:150:240 kg ha^{-1} NPK), T10 (200:50:80 kg ha^{-1} NPK), T11 (200:50:160 kg ha^{-1} NPK), T12 (200:50:240 kg ha^{-1} NPK), T13 (200:100:80 kg ha^{-1} NPK), T14 (200:100:160 kg ha^{-1} NPK), T15 (200:100:240 kg ha^{-1} NPK), T16 (200:150:80 kg ha^{-1} NPK), T17 (200:150:160 kg ha^{-1} NPK), T18 (200:150:240 kg ha^{-1} NPK), T19 (300:50:80 kg ha^{-1} NPK), T20 (300:50:160 kg ha^{-1} NPK), T21 (300:50:240 kg ha^{-1} NPK), T22 (300:100:80 kg ha^{-1} NPK), T23 (300:100:160 kg ha^{-1} NPK), T24 (300:100:240 kg ha^{-1} NPK), T25 (300:150:80 kg ha^{-1} NPK), T26 (300:150:160 kg ha^{-1} NPK), T27 (300:150:240 kg ha^{-1} NPK).

3.3. Quality Parameters

Stevioside (STV) content (%) in stevia dry leaves was significantly modified by different treatments of NPK combinations (Table 3). Treatment T6 (100:100:240 kg ha^{-1} NPK) recorded significantly higher STV content (10.80% of the leaf dry weight) followed by T1 (100:50:80 kg ha^{-1} NPK) (8.20%) which was statistically on par with T20 (300:50:160 kg ha^{-1} NPK) but recorded significantly higher STV content than other treatments and unfertilized pot, while the lowest content of STV (3.35%) was obtained with 100:100:160 kg ha^{-1} NPK (T5). Also, different treatments caused a significant effect on rebaudioside A (Reb A) content, total steviol glycosides (SG) content and total SG yield (Table 3). Highest total SG content (15.05%) was possible with T6 which was on par with T1 and both were superior to remaining treatments and control. Lower content of total SG (8.15%) was observed in T17 (200:150:160 kg ha^{-1} NPK). This treatment recorded about 45.85% and 43.40% lower total SG content in leaf compared with T6 and T1, respectively. However, the maximum content of Reb A (5.60%) was recorded with the application of 200:100:80 kg ha^{-1} NPK (T13) which was followed by T16 (200:150:80 kg ha^{-1} NPK) (4.55%) which was on par with T11 (200:50:160 kg ha^{-1} NPK), T18 (200:150:240 kg ha^{-1} NPK), and T21 (300:50:240 kg ha^{-1} NPK) but recorded significantly higher Reb A content as against all other treatments applied with nutrients and control. T13 recorded about 82.14% higher Reb A content in leaf compared with T20 (300:50:160 kg ha^{-1} NPK) which recorded lower content (1%). Though the highest value of total SG yield (2.13 g $plant^{-1}$) was obtained with 300:100:240 kg ha^{-1} NPK (T24) as compared to other treatments and control, while the lowest yield of total SG (0.34 g $plant^{-1}$) was obtained with the control and was on par with T2 (100:50:160 kg ha^{-1} NPK) and T3 (100:50:240 kg ha^{-1} NPK). The total SG yield was decreased in control than T24 (84.04%).

Table 3. *Effect of NPK fertilization on steviol glycosides of stevia.*

Treatment	Parameters			
	Stevioside (%)	**Rebaudioside A (%)**	**Total SG (%)**	**Total SG (g $plant^{-1}$)**
T0	4.75efghij	2.60ghi	9.60jkl	0.34i
T1	8.20b	4.20bcd	14.40a	0.74h
T2	6.40cde	4.00bcde	11.60cdefg	0.40i
T3	6.55bcd	2.95fgh	10.85defghi	0.48i
T4	6.00cdefgh	3.20efg	10.35ghij	0.74h
T5	3.35j	3.45cdefg	10.75defghi	0.70h
T6	10.80a	2.75ghi	15.05a	0.79h
T7	6.70bcd	3.35defg	11.85bcdef	0.74h
T8	4.60fghij	4.10bcd	10.15hijk	0.71h
T9	5.05cdefghij	1.40jk	8.70lm	1.09g
T10	4.35hij	4.00bcde	9.90hijkl	1.09g
T11	5.15cdefghi	4.50b	11.15cdefgh	1.25defg
T12	4.65efghij	3.20efg	9.25jklm	1.31def
T13	5.35cdefghi	5.60a	13.00b	1.66b
T14	6.65bcd	1.95ij	9.70ijkl	0.87h
T15	6.30cdef	3.75bcdef	11.90bcde	1.39cd
T16	6.70bcd	4.55b	12.95b	1.20efg
T17	4.65efghij	2.20hi	8.15m	0.81h
T18	4.40ghij	4.45b	10.60efghi	1.33de
T19	6.15cdefg	4.00bcde	12.00bcd	1.65b
T20	6.80bc	1.00k	9.15jklm	1.15fg
T21	4.10ij	4.40b	11.05cdefgh	1.34de
T22	5.25cdefghi	2.65ghi	8.95klm	1.52bc
T23	6.15cdefg	3.45cdefg	10.70defghi	1.52bc
T24	5.00defghij	4.15bcd	10.65efghi	2.13a
T25	6.20cdef	4.25bc	12.20bc	1.10g
T26	5.05cdefghij	3.95bcde	10.55fghi	1.21efg
T27	6.10cdefgh	4.00bcde	11.65cdefg	1.53bc

* Means followed by different letters in each column are significantly different (Duncan multiple range test at the 5% significance level).

T0 (Control), T1 (100:50:80 kg ha^{-1} NPK), T2 (100:50:160 kg ha^{-1} NPK), T3 (100:50:240 kg ha^{-1} NPK), T4 (100:100:80 kg ha^{-1} NPK), T5 (100:100:160 kg ha^{-1} NPK), T6 (100:100:240 kg ha^{-1} NPK), T7 (100:150:80 kg ha^{-1} NPK), T8 (100:150:160 kg ha^{-1} NPK), T9 (100:150:240 kg ha^{-1} NPK), T10 (200:50:80 kg ha^{-1} NPK), T11 (200:50:160 kg ha^{-1} NPK), T12 (200:50:240 kg ha^{-1} NPK), T13 (200:100:80 kg ha^{-1} NPK), T14 (200:100:160 kg ha^{-1} NPK), T15 (200:100:240 kg ha^{-1} NPK), T16 (200:150:80 kg ha^{-1} NPK), T17 (200:150:160 kg ha^{-1} NPK), T18 (200:150:240 kg ha^{-1} NPK), T19 (300:50:80 kg ha^{-1} NPK), T20 (300:50:160 kg ha^{-1} NPK), T21 (300:50:240 kg ha^{-1} NPK), T22 (300:100:80 kg ha^{-1} NPK), T23 (300:100:160 kg ha^{-1} NPK), T24 (300:100:240 kg ha^{-1} NPK), T25 (300:150:80 kg ha^{-1} NPK), T26 (300:150:160 kg ha^{-1} NPK), T27 (300:150:240 kg ha^{-1} NPK).

4. Discussions

Data on growth parameters clearly showed that different treatments of NPK combinations had a significant effect on the growth. In this study, all treatments increased growth parameters as compared to absolute control. Maximum plant height and stem diameter were attained by 200:150:80 kg ha^{-1} NPK. Differences in growth may be because of the higher absorption of water and mineral nutrients due to extensive colonization of roots [16]. [13] and [26] reported that N stimulated the leaf production probably due to the increasing production of cytokinin in root tips and their eventual export to the shoot. The results are in accordance with the findings of [3], who reported that plant height was significantly higher with nitrogen, phosphorus, and potassium (400, 200, and 200 kg ha^{-1}, respectively) which were on par with 300, 150, and 100 kg ha^{-1} respectively. [9] also reported increased plant height and number of branches $plant^{-1}$ with nutrient levels of 40:30:45 kg NPK ha^{-1} in sandy loam soils at Bangalore. Increased plant height and number of leaves $plant^{-1}$ with increased levels of N, P and K fertilizers was also reported by [30] in India.

The higher fresh biomass, fresh leaf yield, and dry leaf yield of stevia with higher levels of nitrogen (300 kg ha^{-1}), phosphorus (100 kg ha^{-1}) and potassium (240 kg ha^{-1}) nutrient combination in the present study could be attributed to more number of branches and leaves $plant^{-1}$, and higher leaf area $plant^{-1}$. The higher dry leaf yield and biomass may be also due to the supply of sufficient nitrogen, phosphorus and potassium during the crop growth period. [6] reported that P is an essential component of key molecules such as nucleic acids, phospholipids, and ATP, which are necessary for photosynthesis, energy transfer, carbohydrate, and protein synthesis. A similar increase in dry leaf yield of stevia with NPK combination was also reported by [3]. [38] showed that stevia plants grown at 40 and 60 kg N ha^{-1} produced significantly higher dry leaf yield than at 0 and 20 kg N ha^{-1}. Increased dry leaf yield was also reported by [25] with 105:30:45 kg NPK ha^{-1} as compared to lower doses of NPK under loamy soil in Karnataka, India. Similarly, [23] observed that shortly before or at flowering, production of 1 ton of dry leaves of stevia required 64.6 kg N ha^{-1}, 7.6 kg P ha^{-1} and 56.1 kg K ha^{-1}. [32] reported that the applications of 90 kg N, 40 kg P and 40 kg K ha^{-1} are the best nutritional conditions in terms of dry leaf yield for CSIR-IHBT (Council of Scientific and Industrial Research- Institute Himalayan Bioresource Technology) and RHRS (Regional Horticultural Research Station) conditions. Significantly lower fresh biomass, fresh leaf yield, and dry leaf yield were obtained with the absolute control as against all other treatments applied with nutrients, due to the lowest number of branches and leaves $plant^{-1}$. [29] in Japan experimentally proved that no manuring resulted in lowest leaf yield of stevia. [42] also reported lower dry leaf yield with absolute control without any fertilizer, which was 62 and 63 per cent less as compared to higher levels of nitrogen and phosphorous. In accordance of our results yield of stevia increased significantly with increasing rates of N, P and K up to 60:30:45 kg ha^{-1} per crop with the highest dry leaf yield which was on par with 40:20:30 kg ha^{-1} per crop in sandy loam soils at Bangalore [9]. [5] reported increased biomass and leaf yield due to the application of higher levels of phosphorus and potassium, but no significant effect of higher level of nitrogen in an Andosol with a pH of 4.5 at Canada. Research conducted at Egypt also showed a gradual and significant increase in fresh and dry leaf biomass yields of stevia when nitrogen fertilizer was increased from 10 to 30 kg N ha^{-1} wherein the dry leaves yield increased by 64 per cent compared to lower dose [4]. While, [22] reported increase in leaf yield with moderate application of N, P and K fertilizers in Korea. There are, however, reports that stevia crop shows yield reduction at high rates of fertilizer.

Nitrogen, phosphorus, and potassium contents in stevia leaf at harvest were significantly influenced by the levels of N, P, and K. The contents of N, P, and K were increased with higher doses of N, P, and K, respectively. The higher content of N, P, and K nutrients may be attributed to the adequate quantity and higher availability of these nutrients in the root zone during plant growth period. Also, this increase was generally caused by higher dry leaf yield obtained at the same levels. These findings are in conformity with the results reported by [5] where in higher nutrients content in stevia plant was attributed to the higher availability of nutrients in the root zone. [3] also recorded higher NPK content with higher availability of NPK nutrients. Earlier [38] have also reported that increased supply of nitrogen resulted in increased plant N content by stevia. However, [18] in Japan reported higher nitrogen (1.4%), phosphorus (0.3%) and potassium (2.4%) content in stevia plant at harvest with adequate fertilization. [32] reported that applied N, P and K had little effect in altering the concentration of N, P and K in stevia plant. The absolute control recorded the lowest N, P and K concentrations. It has also been reported that P deficiency reduced absolute root growth of rice (*Oryza sativa* L.) [47].

Stevioside (STV) and total steviol glycosides (SG) contents in stevia leaves were higher with treatment 100:100:240 kg ha^{-1} NPK than all other treatments and control, which may be due to combined effects of moderate levels of nitrogen and phosphorus, and higher level of potassium. The greater STV content in leaf with moderate dose of N may be attributed to the desired dose of photosynthetic pigments. [21] reported that accumulation of steviol glycosides in cells of stevia *in vivo* and *in vitro* was related to the extent of the development of the membrane system of chloroplasts and the content of photosynthetic pigments. [32] reported that stevioside accumulation in leaf was significantly improved by the moderate level of N. However, the maximum content of rebaudioside A (Reb A) was recorded with the application of 200:100:80 kg ha^{-1} NPK. [3] showed that combination of higher levels of nitrogen, phosphorus and, potassium resulted in marginally higher contents of stevioside and rebaudioside A in leaves at harvest as compared to combination of lower levels of these nutrients. [45] from Brazil reported that the deficiency of the major nutrients decreased the stevioside content in the plant. Higher SG yield was obtained with higher levels of nitrogen, phosphorus, and potassium combination, this due to the combined influence of greater nutrient concentrations and dry biomass yield under with those this combination. Similar results were reported by [3] who reported highest stevioside yield and rebaudioside A yield was obtained with higher NPK levels and the lowest stevioside yield and rebaudioside A yield was recorded with the crop applied with no nutrients i.e., absolute control.

5. Conclusion

The results, obtained in the present study, suggest that the stevia growth, yield, and quality are strongly controlled by the exogenous supply of plant nutrition. Therefore, it can be concluded that the application of 300:100:240 kg ha^{-1} NPK is helpful to increase the fresh biomass yield, fresh leaf yield, dry leaf yield, and steviol glycoside yield as compared to other combinations and absolute control. This superior combination also resulted in the considerably higher content of nitrogen in stevia leaf. However, higher phosphorus and potassium contents were obtained with 300:150:80 kg ha^{-1} NPK and 100:50:240 kg ha^{-1} NPK combinations, respectively. The combination of 200:150:80 kg ha^{-1} NPK was found to be more effective for plant height and stem than all other treatments while, the stevioside and total steviol glycoside contents in stevia leaves were higher with 100:100:240 kg ha^{-1} NPK combination. Thus, the combination of 300:100:240 kg ha^{-1} NPK could be considered as an economically optimum level of nutrients for stevia in sandy soil under north-western Moroccan conditions.

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