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EFFECT OF PINCHING AND FERTILIZATION TREATMENTS ON VEGETATIVE GROWTH, SEPAL PRODUCTION AND CHEMICAL COMPOSITION OF ROSELLE (*Hibiscus sabdariffa* L.)

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ABSTRACT

This study was conducted in the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University. Giza, during the two successive seasons of 1999 and 2000, with the aim of investigating the effect of manual and chemical pinching (using foliar kinetin application), as well as NPK and biofertilization treatments, on growth, sepal production and chemical composition of roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*, cv. Sabahia 17, dark color).

Roselle (*Hibiscus sabdariffa* L.) plants were either: (1) left unpinched, (2) pinched manually (twice: one month after planting, and one month later), or (3) pinched chemically using foliar spray applications of kinetin at concentrations of 15, 30 or 45 ppm (3 applications, at 15 day intervals, starting 30 days from planting). Also, the plants were supplied with NPK fertilization [using ammonium sulphate (20.5% N) at 400 kg/fed., calcium superphosphate (15.5% P_2O_5) at 150 kg/fed., and potassium sulphate (48% K_2O) at 50 kg/ fed.], or with biofertilization (alone or in combination with NPK fertilization at 100% or 50% of the above rates). Plants supplied with 100% NPK (with no biofertilization) were used as the control. In treatments including biofertilization, the biofertilizer (a mixture of vermiculite and *Azotobacter chroococcum*, at a concentration of 10^9 bacteria/g vermiculite) was mixed with the seeds prior to planting at a rate 500 g biofertilizer/250 g seeds. In addition, biofertilization was applied after planting as a liquid top dressing 3 times/ season (at 30 day intervals). The liquid inoculum consisted of a mixture of molasses and *Azotobacter chroococcum*.

Among the different manual and chemical pinching treatments, application of kinetin at 15 ppm gave the highest values for most of the studied growth and yield characteristics (plant height, number of branches, leaves and fruits/plant, as well as fresh and dry weights of leaves and sepals/plant). Kinetin at 15 ppm also increased the leaf contents of total chlorophyll, carotenoids, total carbohydrates, N, P and K, as well as the total acidity and anthocyanin contents of the sepals. Supplying the plants with 100% NPK + biofertilization gave the highest values for number of branches, leaves and fruits/plant, fresh and dry weights of leaves/plant, the contents of total carbohydrates, P and K in the leaves, as well as the anthocyanin content in the sepals, compared to all other fertilization treatments. However, using 50% NPK + biofertilization gave the highest fresh and dry weights of sepals/plant, and insignificantly increased the number of branches/plant, compared to those formed on plants fertilized with 100% NPK alone (control plants), i.e. biofertilization reduced the need for chemical NPK fertilization (for branching and sepals production) by at least 50%. Fertilization with 50% NPK + biofertilization also gave the highest contents of total chlorophyll and N in the leaves, as well as the highest total acidity and total soluble solids contents in the sepals. Moreover, combining kinetin at 15 ppm with application of 50% NPK + biofertilization gave the best results, in terms of number of branches and fruits/plant, fresh and dry weights of sepals/plant, the total

carbohydrates content in leaves, as well as the content of total soluble solids in the sepals, compared to any other combination of pinching and fertilization treatments.
Keywords: *Azotobacter chroococcum*, biofertilization, cytokinin, fertilization, *Hibiscus sabdariffa*, kinetin, NPK, pinching, roselle.

INTRODUCTION

Hibiscus sabdariffa, L. (roselle, Jamaica sorrel, red sorrel, karkade) is one of the most important plants of the Malvaceae family. Roselle is grown mainly for its edible acid red fleshy calyces and involucres. The calyx is used in drinks and for making jelly [Bailey (1951), Everett (1981) and Al-Wandawi *et al.* (1984)]. The fleshy sepals contain pigments (especially anthocyanin), a mixture of organic acids (malic, citric, hibiscic, and tartaric acid), and has antibacterial, antifungal and diuretic activities [Khafaga and Koch (1980), Caceres *et al.* (1987)]. Roselle has an acid tonic taste, and is well tolerated by patients with fever (Ahmed *et al.* 1998). The sour tea (made of roselle sepals) was found to reduce hypertension (Faraji and Tarkhani, 1999). The soft drink of *Hibiscus sabdariffa* was studied for antioxidant activity (Jong *et al.*, 2000). Ethanol extracts (80 %) of roselle flowers have been used for antimutagenic and chemopreventive activity. In the *Salmonella* mutation assay, the extract (12.5 mg/ plate) reduced about 60-90% of the mutagenecity induced by several chemicals (Chewonarian *et al.*, 1999). Moreover, protocatechuic acid (PCA), a phenolic compound isolated from dried flowers of *H. sabdariffa*, is known to have antioxidant and antitumour promotion effects (Tsuihwa *et al.*, 2000).

In a plant like roselle, promotion of branching may result in an increase in the number of flowers and, consequently, an increase in the yield of sepals. This can be achieved by manual removal of the apical bud (manual pinching). In fact, manual pinching was proved to be effective in increasing the number of branches and flowers, as well as increasing fruit weight and sepal weight in *Hibiscus sabdariffa* plants [Khafaga et *al.* (1980), and Omer *et al.* (1997)]. However, manual pinching is not a practical procedure in commercial production fields. Foliar application of chemicals (such as cytokinins), which is a relatively easier and simpler practice, may replace manual pinching as a method for promoting branching. It has been reported that the application of cytokinins to the buds of intact plants forces them to grow out (Sachs and Thimann, 1964). Cytokinins (such as BA and kinetin) have been found to promote branching and increase yields of several important crops [Zayed *et al.* (1985) on *Hibiscus sabdariffa*, Runkova (1985) on *Helenium, Phlox, Dahlia* and *Calendula* plants, Hedin *et al.* (1988) and Sawan *et al.* (2000) on cotton, and Ibrahim and Tarraf (2000) on *Foeniculum vulgare*].

Chemical fertilization is another important cultural practice that has a considerable influence on growth, yield and chemical composition of different crops, including *Hibiscus sabdariffa* (Selim *et al.*, 1993). However, organically-grown agricultural products are considered to be healthier, and cause less risk to the environment, since their tissues contain lower levels of chemical residues. In bio-organic farming systems, chemical fertilization is often replaced (at least partly) by biofertilization, using different nitrogen-fixing bacteria. *Azotobacter* are free living bacteria that are capable of nonsymbiotic nitrogen fixation (Burris, 1976). In addition to their N-fixing proficiency, the beneficial effects of *Azotobacter* bacteria were also attributed to their ability to produce antibacterial and antifungal compounds, and growth regulators (Pandey and Kumar, 1989). Studies conducted by a number of researchers have shown that inoculation with *Azotobacter* bacteria (including *A. chroococcum*) improved the growth and yield characteristics in several important crops, including cotton [Patil and Patil (1984), Chitriv and Wangikar (1986), Shende *et al.* (1988), Kurdikeri and Kurdikeri (1988), Pandey and Kumar (1989), Prasad and Prasad (1994)], okra [Mishra and Patjoshi (1995) and Patil *et al.* (2000)], *Cymbopogon martinii* (Maheshwari *et al.*, 1995), and tomato (Puertas and Gonzalez, 1999). In some of these studies, the favourable effect of *Azotobacter* bacteria was more pronounced when biofertilization was combined with chemical fertilization (especially N fertilization).

In this study, the effect of manual and chemical pinching (using foliar kinetin application), as well as chemical NPK fertilization and biofertilization using *Azotobacter chroococcum* on growth, sepal yield and chemical composition of *Hibiscus sabdariffa* L. var. *sabdariffa* was investigated.

MATERIALS AND METHODS

This study was conducted in the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 1999 and 2000, with the aim of investigating the effect of manual pinching and foliar kinetin application (referred to as chemical pinching), as well as NPK and bio-fertilization treatments, on growth, sepal production and chemical composition of roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*, cv. Sabahia 17, dark color).

On 15^{th} and 21^{st} May, 1999 and 2000 (in the first and the second seasons, respectively), seeds of *Hibiscus sabdariffa* var. *sabdariffa* were directly sown on ridges in plots (plot dimensions were 1.0 x 1.6 meters, with 3 ridges/ plot, and 60 cm between ridges), at a spacing of 30 cm distance between plants. The physical and chemical characteristics of the soil in the experimental area are shown in Table (1). Farmyard manure (FYM) was incorporated into the soil prior to planting, at a rate equivalent to 20 m^3/fed. The layout of the experiment was a "Randomized Complete Blocks Design", with 20 treatments (4 fertilization treatments x 5 pinching treatments) and three replicates (plots)/ treatment.

After planting, the plants were either left intact (unpinched), were pinched manually (twice: one month after planting, and one month later), or were sprayed 3 times (at 15 day intervals, starting 30 days from planting) using kinetin at concentrations of 15, 30 or 45 ppm. Also, the plants were supplied with NPK fertilization [using ammonium sulphate (20.5% N) at 400 kg/fed., calcium superphosphate (15.5% P_2O_5) at 150 kg/fed., and potassium sulphate (48% K_2O) at 50 kg/ fed.] or biofertilization (using a mixture of vermiculite and *Azotobacter chroococcum*, at a concentration of 10^9 bacteria/g vermiculite) alone or in combination with NPK fertilization at 100 % or 50% of the above rates. The mineral fertilizers were combined and added as a basal dressing. The above rates were divided into two doses: the first dose was added after one month from planting, and the second one was added 45 days later. Plants supplied with 100% NPK fertilization (with no biofertilization) were used as the control. In treatments including the use of biofertilization, the biofertilizer was mixed with the seeds prior to planting (at a rate of 250 g seeds + 500 g biofertilizer). After planting, the biofertilizer was applied as a liquid top dressing 3 times/ season (at 30 days intervals), with the first application added after 30 days from planting. The liquid inoculum was prepared using a mixture of molasses and *Azotobacter chroococcum* at the concentration of 10^9 bacteria/ ml molasses. Prior to application, this mixture was diluted to 1/ 20 of the original concentration. Common cultural practices were followed, including regular irrigation (as needed) and manual weed control.

Table (1): Physical and chemical analysis of the experimental soil.

Physical analysis			
Clay %	**Silt %**	**Sand %**	**Texture**
42.96	8.28	48.76	Sandy clay

Chemical analysis												
pH	***E.C. (dS/m)**	**$CaCO_3$ (%)**	**Soluble Cations (meq./L)**				**Soluble Anions (meq./L)**			**Available elements (ppm)**		
			Na^+	**K^+**	**Ca^{++}**	**Mg^{++}**	**HCO_3^-**	**Cl^-**	**SO_4^{--}**	**N**	**P**	**K**
7.60	0.79	2.37	2.43	1.20	3.54	0.60	1.30	2.77	3.70	35.5	65.1	675

***E. C.: Electrical conductivity**

At the end of each season, (on 25th and 29th September, 1999 and 2000 in the first and second seasons, respectively), the plants were harvested by cutting the stems 5 cm above the soil. The data recorded on vegetative growth included plant height (cm), number of branches/plant, number of leaves / plant, fresh and dry weights of leaves and sepals/plant (g), as well as number of fruits/plant. The above data were subjected to an analysis of variance (ANOVA), and the means were compared using the "Least Significant Difference (LSD)" test at the 0.05 level, as described by Steel and Torrie (1980).

Chemical analysis of fresh leaf samples was carried out to determine the leaf pigments (total chlorophyll and carotenoids) content (using the method described by Saric *et al.*, 1967). Also, leaf samples were oven-dried at a temperature of 70° C for 24 hours, until a constant weight was obtained, as recommended by the A.O.A.C. (1965). The content of total carbohydrates in the dried leaves were determined as described by Dubois *et al.* (1956).

In addition, dry leaf samples were digested for extraction of minerals (using the method described by Piper, 1947), and the content of nitrogen in the extract was determined using the modified Micro-Kjeldahl method (as described by Pregl, 1945), while the phosphorus content was determined colorimeterically using the method recommended by Jackson (1967), and the potassium content was determined using an atomic absorption, flame-photometer (Philips, model PU 9100 X), as recommended by Chapman and Pratt (1961).

Samples of calyces and epicalyces were air-dried until a constant weight was obtained. The anthocyanin content in the dried sepals was determined according to the method described by Fulker and Francis (1968). An aqueous sepal extract was prepared, and its pH value was determined using a pH-meter, as recommended by Diab (1968). The percentage of total acidity in sepals (as citric acid), was determined using the method described by the A.O.A.C. (1965), while the total soluble solids content (%) in calyces and epicalyces was determined using a referactometer, according to the method described by the A.O.A.C. (1965).

RESULTS AND DISCUSSION

I. Effect on vegetative growth

1. Plant height

Data recorded in the two seasons (Table 2) on roselle (*Hibiscus sabdariffa* L. var. sabdariffa) plants show that manual pinching led to a significant decrease in plant height, compared to the control, or to plants receiving the different kinetin treatments. This conclusion is similar to that reached by Lee and Kwack (1994), who stated that one hand-pinching treatment was enough to retard shoot growth in *H. syriacus* cv. Hanol-tanshjm. On the other hand, spraying kinetin at the lowest concentration (15 ppm) significantly increased plant height. This increase may be attributed to the role of cytokinins in cell enlargement and tissue differentiation, as stated by Leopold and Kriedmann (1975). However, a further increase in the concentration of kinetin from 15 to 30 or 45 ppm caused a steady decrease in plant height.

Regarding the effect of fertilization, the data presented in Table (2) show that the response of *Hibiscus sabdariffa* to the different fertilization treatments varied from one season to the other. In the first season, there was a steady increase in mean plant height with the decrease in NPK rate and/or the addition of biofertilization. In contrast, the results recorded in the second season showed that reducing the NPK rate caused a reduction in plant height, regardless of the use of bio-fertilizers.

The data in Table (2) also show that, in both seasons, combining kinetin at 15 ppm with fertilization using 100% NPK + biofertilizer resulted in the tallest plants. These plants were significantly taller than unpinched plants fertilized with 100% NPK (control), or plants receiving most of the other combinations of pinching and fertilization treatments. In contrast, manual pinching, combined with fertilization using 100 % NPK + bio-fertilizer led to the shortest plants.

Table (2): Effect of manual pinching, kinetin, NPK and biofertilization treatments on plant height, number of branches/plant and number of leaves/plant in roselle (*Hibiscus sabdariffa*) plants in the 1999 and 2000 seasons.

*Fertilization treatments (B)	First season (1999)						Second season (2000)					
	Pinching methods (A)					Means	Pinching methods (A)					Means
	Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)		Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)	
	Plant height (cm)											
100% NPK	112.10	79.83	131.00	109.33	108.33	**108.10**	109.83	86.99	113.50	110.83	106.80	**105.60**
100% NPK + BF	122.00	76.21	132.72	120.53	109.29	**112.20**	115.11	62.30	125.13	113.20	111.50	**105.40**
50% NPK + BF	124.29	95.33	115.50	113.75	124.00	**114.60**	97.01	80.10	115.33	112.66	99.12	**100.80**
BF	116.50	90.05	124.01	124.67	120.10	**115.10**	105.17	74.30	110.44	113.55	110.10	**102.70**
Means	**118.70**	**85.36**	**125.80**	**117.10**	**115.40**	---	**106.78**	**75.92**	**116.10**	**112.60**	**106.90**	---
LSD (0.05)	A: 3.88		B: 3.47		A X B: 7.77		A: 3.13		B:2.80		A X B: 6.25	
	Number of branches/plant											
100% NPK	9.30	10.91	10.30	8.80	8.50	**9.56**	7.50	9.66	9.00	7.00	6.00	**7.83**
100% NPK + BF	8.71	11.79	12.00	11.00	10.01	**10.70**	6.99	10.33	10.62	9.70	8.00	**9.13**
50% NPK + BF	10.11	11.30	12.40	8.02	8.90	**10.15**	8.60	10.00	10.80	5.79	7.40	**8.52**
BF	6.40	7.95	8.60	7.71	6.40	**7.41**	5.00	5.73	6.95	5.30	5.11	**5.62**
Means	**8.63**	**10.50**	**10.83**	**8.88**	**8.45**	---	**7.02**	**8.93**	**9.34**	**6.95**	**6.63**	---
LSD (0.05)	A: 1.23		B: 1.10		A X B: N.S.		A: 1.37		B: 1.22		A X B: N.S.	
	Number of leaves/plant											
100% NPK	74.11	74.29	100.41	82.30	89.39	**84.10**	70.39	71.40	75.41	70.38	77.39	**72.99**
100% NPK + BF	87.10	89.30	104.25	92.19	90.17	**92.60**	72.30	75.40	85.29	76.30	75.20	**76.90**
50% NPK + BF	81.30	85.31	82.29	78.21	70.40	**79.50**	71.40	85.50	85.52	71.38	67.30	**76.22**
BF	60.60	65.43	78.56	75.50	69.49	**69.92**	59.56	61.50	75.19	69.20	63.31	**65.75**
Means	**75.78**	**78.58**	**91.38**	**82.05**	**79.86**	---	**68.41**	**73.45**	**80.35**	**71.82**	**70.80**	---
LSD (0.05)	A: 3.23		B: 2.89		A X B: 6.45		A: 2.00		B: 1.79		A X B: 4.01	

*** 100% NPK = Ammonium sulphate at 400 kg/fed. + calcium superphosphate at 150 kg/fed. + potassium sulphate at 50 kg/fed.**
BF = Biofertilization using *Azotobacter chroococcum* as a preplanting seed treatment and post planting top dressings.

2. Number of branches/plant

The data in Table (2) show that in both seasons, manual pinching resulted in a significant increase in the number of branches/plant, compared to the control. These results are in agreement with the findings of Omer *et al.* (1997) who mentioned that pinching of *Hibiscus sabdariffa* plants increased the number of branches.

Spraying kinetin at 15 ppm also gave significantly more branches than the unpinched control plants. This promotion of branching in plants sprayed with kinetin can be attributed to the role of cytokinin (kinetin) in forcing the buds of intact plants to grow out, as mentioned by Sachs and Thimann (1964). Also, Taiz and Zeiger (1998) reported that cytokinins induce bud formation. On the other hand, raising the kinetin concentration to 30 or 45 ppm caused a steady reduction in the number of branches formed on roselle plants. In both seasons, plants sprayed with kinetin at 30 or 45 ppm gave values which were insignificantly different than those obtained from control plants.

Regarding the effect of fertilization on branching, it was found that plants fertilized with 100% NPK + biofertilization had significantly more branches than those fertilized using 100% NPK alone. Reducing the NPK rate to 50%, combined with biofertilization, gave a number of branches that was insignificantly higher than that found on control plants (100% NPK), i.e. combining NPK fertilization with biofertilization reduced the need for chemical fertilization by 50%. On the other hand, plants supplied with biofertilization only had significantly fewer branches than those supplied with the other three fertilization treatments.

The combination of chemical pinching using kinetin at 15 ppm with fertilization using 50% NPK + biofertilization gave the highest number of branches/plant, compared to all other combinations of pinching and fertilization treatments.

3. Number of leaves/plant

The results presented in Table (2) show that in both seasons, unpinched control plants gave the lowest number of leaves. On the other hand, the different manual and chemical pinching treatments increased the formation of leaves by *Hibiscus sabdariffa* plants. Among the different treatments, kinetin at 15 ppm was the most effective one, giving significantly more leaves than any other treatment (in both seasons). Raising the kinetin concentration from 15 to 30 or 45 ppm caused a steady reduction in the values recorded in the two seasons. In both seasons, the lowest numbers of leaves were obtained from plants that were pinched manually (in the first season) or that were treated with kinetin at 45 ppm (in the second season).

Regarding the effect of the fertilization treatments on the formation of leaves by *Hibiscus sabdariffa* plants, it was noticed that combining conventional NPK fertilization at the recommended rate (100% NPK) with the application of biofertilization gave higher values in both seasons, compared to plants receiving 100% NPK alone, or plants receiving any other fertilization treatment. In contrast, the use of biofertilization only gave the lowest values recorded in both seasons. The results recorded with 50% NPK +

biofertilization differed from one season to the other. In the first season, plants supplied with this treatment gave fewer leaves than the control, but in the second season this treatment gave significantly more leaves than those produced by control plants.

The data in Table (2) also show the effect of different combinations of fertilization treatments and pinching methods on the number of leaves/plant. Combining 100% NPK + biofertilization with the use of kinetin at 15 ppm gave the highest number of leaves in the first season, while in the second season, the combination of 50% NPK + biofertilization with kinetin at 15 ppm gave the highest value.

4. Fresh and dry weights of leaves/plants

From the results presented in Table (3), it is clear that manual pinching significantly increased the fresh and dry weights of leaves/plant, compared to the unpinched control plants. The fresh and dry weights of leaves were also increased significantly by chemical pinching using kinetin at 15 or 30 ppm. In both seasons, plants treated with kinetin at 15 ppm gave significantly higher values, compared to those obtained from unpinched control plants, or plants receiving any other manual or chemical pinching treatment. These results are in agreement with the findings of Ibrahim and Tarraf (2000), who mentiond that spraying Egyptian fennel (*Foeniculum vulgare* Mill. var. *dulce*) plants with kinetin had a significant positive effect on the fresh and dry weights of leaves. However, the recorded values were decreased steadily by raising the kinetin concentration. Accordingly, when kinetin was applied at the highest concentration (45 ppm) it gave significantly lower values, compared to the control or to plants receiving all the other pinching treatments.

Regarding the effect of the fertilization treatments, the data in Table (3) show that combining chemical fertilization (100% or 50% NPK) with biofertilization caused a significant increase in the fresh and dry weights of leaves/plant, compared to values obtained from plants fertilized with 100% NPK alone (the control). In both seasons, the most effective fertilization treatment for increasing the fresh and dry weights of leaves was the application of 100% NPK + biofertilization, which gave significantly higher values than any other fertilization treatment. On the other hand, plants supplied with biofertilization only gave significantly lower values, compared to all the other fertilization treatments.

The data presented in Table (3) also show that significant differences were detected between the values obtained from plants receiving different combinations of pinching and fertilization treatments. In both seasons, the highest values were obtained from plants pinched chemically using kinetin at 15 ppm, combined with fertilization using 100% NPK + biofertilization. On the other hand, the lowest values were obtained from unpinched plants fertilized with biofertilization only.

Table (3): Effect of manual pinching, kinetin, NPK and biofertilization treatments on the fresh and dry weights of leaves/plant, and the number of fruits/plant in roselle (*Hibiscus sabdariffa*) plants in the 1999 and 2000 seasons.

*Fertilization treatments (B)	First season (1999)						Second season (2000)					
	Pinching methods (A)					Means	Pinching methods (A)					Means
	Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)		Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)	
	Fresh weight of leaves (g/plant)											
100% NPK	178.10	261.00	345.50	251.13	220.70	**251.29**	160.30	238.40	305.10	225.50	209.13	**227.70**
100% NPK + BF	291.40	215.50	347.40	300.90	247.96	**280.63**	267.30	195.80	320.10	288.60	219.90	**258.30**
50% NPK + BF	259.30	341.60	342.50	227.00	200.50	**274.18**	228.10	298.10	300.17	210.70	180.61	**243.50**
BF	159.40	165.00	225.80	191.24	168.40	**181.97**	155.40	158.10	209.10	171.21	158.00	**170.40**
Means	**222.05**	**245.78**	**315.30**	**242.57**	**209.39**	---	**202.78**	**222.60**	**283.62**	**224.00**	**191.91**	---
LSD (0.05)	A: 3.63		B: 3.24	A X B: 7.25			A: 3.42		B: 3.06	A X B: 6.83		
	Dry weight of leaves (g/plant)											
100% NPK	25.90	41.80	61.10	39.50	35.00	**40.66**	23.00	40.91	56.92	36.50	33.10	**38.09**
100% NPK + BF	52.30	34.10	61.84	54.90	39.00	**48.43**	47.62	31.09	58.25	51.30	35.30	**44.71**
50% NPK + BF	41.03	60.00	60.20	36.10	29.30	**45.33**	37.01	54.50	55.10	32.90	29.00	**41.70**
BF	21.40	24.00	35.90	26.40	25.01	**26.54**	19.20	21.40	33.40	26.03	22.10	**24.43**
Means	**35.16**	**39.98**	**54.76**	**39.23**	**32.08**	---	**31.71**	**36.98**	**50.92**	**36.69**	**29.88**	---
LSD (0.05)	A: 1.89		B: 1.69	A X B: 3.78			A: 1.67		B: 1.50	A X B: 3.35		
	Number of fruits/plant											
100% NPK	13.10	15.64	17.51	12.50	14.80	**14.71**	11.40	12.00	16.11	11.30	11.67	**12.50**
100% NPK + BF	12.48	16.80	17.80	17.00	16.90	**16.20**	10.20	13.00	16.60	13.60	12.71	**13.22**
50% NPK + BF	16.31	10.23	18.00	10.40	10.00	**12.99**	12.00	9.50	16.80	9.80	8.60	**11.34**
BF	9.68	9.50	17.31	9.79	12.10	**11.68**	8.01	8.02	16.00	8.50	10.01	**10.11**
Means	**12.89**	**13.04**	**17.66**	**12.42**	**13.45**	---	**10.40**	**10.63**	**16.38**	**10.80**	**10.75**	---
LSD (0.05)	A: 1.83		B: 1.63	A X B: 3.65			A: 1.68		B: 1.50	A X B: N.S.		

*** 100% NPK = Ammonium sulphate at 400 kg/fed. + calcium superphosphate at 150 kg/fed. + potassium sulphate at 50 kg/fed.**

BF = Biofertilization using *Azotobacter chroococcum* as a preplanting seed treatment and post planting top dressings.

II. Fruits and sepals production

1. Number of fruits / plant

The data presented in Table (3) show that, in both seasons, manual pinching gave insignificantly more fruits per plant, compared to unpinched control plants. Also, application of kinetin at 30 or 45 ppm had no significant effect on the number of fruits formed by roselle plants, compared to the control. On the other hand, the lowest kinetin concentration (15 ppm) gave a significantly higher number of fruits in both seasons, compared to any other pinching treatment (including the control). It can be concluded that application of kinetin at a relatively low concentration promoted fruit-set in roselle plants. A similar conclusion was reached by Sawan *et al.* (2000) who found that application of 5 ppm kinetin to Egyptian cotton (*Gossypium barbadense*) at 60 and 75 days after sowing during the beginnig of the bolling stages led to a significant increase in the number of open bolls / plant.

The results recorded in the two seasons (Table 3) also show that plants fertilized with a combination of 100% NPK and biofertilization produced an insignificantly higher number of fruits, compared to those fertilized with 100% NPK alone. On the other hand, supplying the plants with biofertilization alone, or with 50% NPK + biofertilization, decreased the number of fruits produced by roselle plants in the two seasons, compared to the control plants (fertilized with 100% NPK alone). This reduction was significant in most cases.

Regarding the interaction between pinching and fertilization treatments, the data in Table (3) show that spraying the plants with kinetin at 15 ppm, combined with fertilization using 50% NPK + biofertilization gave the greatest values in both seasons, compared to all other treatment combinations.

2. Fresh and dry weights of sepals/plant

The results shown in Table (4) reveal that in both seasons, manual pinching caused a reduction in the fresh and dry weights of sepals/plant, compared to the control plants. In most cases, this reduction was insignificant, except in plants pinched manually in the first season, which gave sepals with a significantly lower fresh weight than that of unpinched control plants. In contrast, the different kinetin treatments had a generally favourable effect on the production of fresh or dry sepals by roselle plants, with all kinetin concentrations giving higher values than the control. In both seasons, kinetin at 15 ppm was the most effective treatment for increasing sepals production, giving significantly higher values than any other pinching treatment, including the control. In contrast, the medium concentration (30 ppm) was the least effective kinetin treatment for increasing the fresh and dry weights of sepals/plant. In both seasons, plants receiving this treatment (kinetin at 30 ppm) gave insignificantly heavier fresh sepals than those produced by unpinched control plants, whereas the dry weight of sepals was significantly increased by this treatment in the first season only, compared to the control.

Table (4): Effect of manual pinching, kinetin, NPK and biofertilization treatments on fresh and dry weights of sepals/plant in roselle (*Hibiscus sabdariffa*) plants in the 1999 and 2000 seasons.

*Fertilization treatments (B)	First season (1999)						Second season (2000)					
	Pinching methods (A)					Means	Pinching methods (A)					Means
	Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)		Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)	
	Fresh weight of sepals (g/plant)											
100% NPK	41.95	31.80	62.40	42.81	47.19	**45.16**	39.61	35.70	61.67	40.12	41.87	**43.79**
100% NPK + BF	45.06	43.15	65.18	50.80	58.33	**52.50**	40.70	40.50	64.13	44.51	50.88	**48.14**
50% NPK + BF	53.63	55.77	71.50	49.15	41.63	**54.34**	47.12	49.71	66.60	43.70	39.73	**49.37**
BF	29.00	30.10	60.13	30.99	42.11	**38.47**	35.10	32.11	59.44	36.00	39.80	**40.49**
Means	**42.32**	**40.21**	**64.80**	**43.44**	**47.32**	---	**40.63**	**39.51**	**62.96**	**41.08**	**43.07**	---
LSD (0.05)	A: 1.97		B: 1.76		A X B: 3.94		A: 1.98		B: 1.77		A X B: 3.96	
	Dry weight of sepals (g/plant)											
100% NPK	4.20	3.90	7.47	4.83	6.01	**5.28**	4.00	2.22	7.39	4.91	5.10	**4.72**
100% NPK + BF	5.03	4.91	7.49	6.33	7.30	**6.21**	5.00	4.97	8.03	6.12	7.10	**6.24**
50% NPK + BF	6.50	6.61	8.51	6.21	4.64	**6.49**	6.70	7.00	8.23	6.00	4.00	**6.39**
BF	2.13	1.89	7.38	3.86	4.70	**3.99**	2.01	1.24	7.28	2.30	4.40	**3.45**
Means	**4.47**	**4.33**	**7.71**	**5.31**	**5.66**	---	**4.43**	**3.86**	**7.73**	**4.83**	**5.15**	---
LSD (0.05)	A: 0.60		B: 0.54		A X B: 1.21		A: 0.66		B: 0.59		A X B: 1.33	

* 100% NPK = Ammonium sulphate at 400 kg/fed. + calcium superphosphate at 150 kg/fed. + potassium sulphate at 50 kg/fed.
BF = Biofertilization using *Azotobacter chroococcum* as a preplanting seed treatment and post planting top dressings.

In both seasons, fertilization of roselle plants with 100% NPK + biofertilization or 50% NPK + biofertilization significantly increased the fresh and dry weights of sepals/plant, compared to that of plants fertilized with 100% NPK alone. The highest values recorded in the two seasons were obtained from plants supplied with 50% NPK + biofertilization. On the other hand, using biofertilization alone significantly decreased the fresh weight of sepals, compared to the control. The above results are in agreement with the findings of Mahmoud and Amara (2000) on tomato. They reported that the best results, in terms of growth parameters and fruit yield, were obtained when the plants were treated with a combination of biofertilization using *Bacillus subtilis* and *B. megatherium* (*B. megaterium*), and chemical fertilization using 50% of the recommended NPK dose.

It is also clear from the data in Table (4) that the best combination of pinching and fertilization treatments (in terms of fresh or dry sepals production) was chemical pinching using kinetin at 15 ppm, combined with 50% NPK + biofertilization. In most cases, the values obtained from plants receiving this treatment combination were significantly higher than those obtained from plants receiving any other treatment combination. Moreover, it is clear from the statistical analysis of the data that treatments which included using kinetin at 15 ppm, combined with any of the four fertilization treatments, were generally more effective for increasing the dry weight of sepals, compared to any other combination of pinching and fertilization treatments.

The increase in vegetative parameters and in the main yield of roselle plants as a result of the different treatments, compared to control plants, may be due to the important role of N (either applied by chemical fertilization, or from N-fixation by bio-fertilizers), P, K and kinetin in the physiological processes within the plant, which in turn affect the growth of the vegetative parts and root system, as well as the fruit and sepal production. Nitrogen is the mineral element which serves as a constituent of many plant cell components, including amino acids and nucleic acids. Phosphorus is an integral component of important compounds of plant cells, including the sugar-phosphate intermediates of respiration and photosynthesis, and the phospholipids that make up plant membranes. It is also a component of nucleotides used in plant energy metabolism and in DNA and RNA. Potassium plays an important role in regulation of the osmotic potential of plant cells. It also activates many enzymes involved in respiration and photosynthesis (Taiz and Zeiger, 1998). Also, the favourable effect of kinetin (especially at 15 ppm) on several growth and yield characteristics were explained by Leopold and Kriedmann (1975), who mentioned that cytokinins participate in cell enlargement and tissue differentiation.

III. Plant chemical composition

1. Leaf chemical composition

a. Pigment content

- Total chlorophylls (a+ b) content

The results recorded in the two seasons (Table 5) show that manual pinching increased the total chlorophylls content of the fresh roselle leaves, compared to the control plants. Also, using kinetin at 15 or 30 ppm increased the total chlorophylls in both seasons, compared to control plants. This conclusion is in agreement with the findings of Wilkins (1984), who reported that a remarkable effect of externally applied cytokinins is their ability to delay the rate of chlorophyll disappearance and protein degradation, which usually accompanies the senescence process in leaves. Also Khokhlova (1977) mentioned that the major effect of cytokinins on chloroplast structure (the site of photosynthesis) appears to be an increase in the internal membrane system. In addition, Taiz and Zeiger (1998) found that cytokinins result in much greener leaves, with higher chlorophyll levels.

Fertilizing roselle plants with biofertilization, alone or in combination with NPK at 100% or 50%, led to an increase in the content of total chlorophylls, compared to that of plants supplied with 100% NPK alone. Among the different fertilization treatments, supplying the plants with 50% NPK + biofertilization was the most effective fertilization treatments for increasing the total chlorophylls content.

Regarding the effect of the different combinations of pinching and fertilization treatmenst on the total chlorophylls content, the data in Table (5) show that in the first season, plants pinched manually and fertilized with 50% NPK + biofertilization had the highest content of total chlorophylls, compared to plants receiving any other combination of pinching and fertilization treatmenst. However, in the second season, the highest content of total chlorophylls was obtained from plants pinched chemically using kinetin at 15 ppm, and fertilized with 50% NPK + biofertilization.

- Total carotenoids content

The results presented in Table (5) show the effect of different pinching and fertilization treatments on the content of carotenoids in leaves of roselle (*Hibiscus sabdariffa* L.) plants. Manual pinching gave different results in the two seasons. In the first season, plants pinched manually had a lower carotenoids content than that of control plants, but in the second season, manual pinching increased the carotenoids content, compared to the control. On the other hand, spraying the plants with kinetin at 15 ppm gave the highest carotenoids content, compared to values obtained with any other pinching treatment. Raising the kinetin concentration from 15 to 30 or 45 ppm resulted in a steady reduction in the carotenoids content. With kinetin at 30 ppm, the recorded values were still higher than those of control plants, but at the highest kinetin concentration (45 ppm), the recorded values were lower than those obtained from control plants.

The data in Table (5) also show that the effect of the different fertilization treatments differed from one season to the other. In the first season, all the fertilization treatments which included application of the bio-fertilizer (100% NPK + biofertilization, 50% NPK + biofertilization, or biofertilization alone) increased the carotenoids content, compared to the control, especially when bio-frtilization was applied alone, giving the highest mean value in the first season. In contrast, all the treatments which included biofertilization decreased the carotenoids content in the second season, especially when biofertilization was added alone, giving the lowest value in the second season.

Table (5): Effect of manual pinching, kinetin, NPK and biofertilization treatments on the contents of total chlorophyll (a + b), carotenoids and total carbohydrates in leaves of roselle (*Hibiscus sabdariffa*) plants in the 1999 and 2000 seasons.

*Fertilization treatments (B)	First season (1999)						Second season (2000)					
	Pinching methods (A)					Means	Pinching methods (A)					Means
	Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)		Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)	
	Total chlorophyll (a + b) content (mg/g fresh matter)											
100% NPK	0.970	1.185	0.952	0.908	0.771	**0.957**	1.298	1.438	1.281	0.953	1.255	**1.245**
100% NPK + BF	1.087	1.140	1.120	1.007	0.767	**1.024**	1.565	1.595	1.292	1.673	1.282	**1.481**
50% NPK + BF	1.022	1.340	1.300	1.285	1.149	**1.219**	1.713	2.111	2.146	2.021	2.117	**2.022**
BF	1.108	0.905	1.070	1.248	1.226	**1.111**	1.324	1.285	1.325	1.396	1.680	**1.402**
Means	**1.047**	**1.142**	**1.110**	**1.112**	**0.978**	---	**1.475**	**1.607**	**1.511**	**1.511**	**1.583**	---
	Carotenoids content (mg/g fresh matter)											
100% NPK	0.322	0.200	0.249	0.245	0.242	**0.252**	0.489	0.413	0.442	0.382	0.384	**0.422**
100% NPK + BF	0.255	0.208	0.335	0.330	0.217	**0.269**	0.327	0.327	0.487	0.457	0.392	**0.398**
50% NPK + BF	0.255	0.340	0.346	0.206	0.207	**0.271**	0.307	0.498	0.490	0.311	0.241	**0.369**
BF	0.258	0.276	0.319	0.320	0.200	**0.275**	0.317	0.398	0.328	0.440	0.358	**0.368**
Means	**0.272**	**0.256**	**0.312**	**0.275**	**0.216**	---	**0.360**	**0.409**	**0.437**	**0.397**	**0.344**	---
	Total carbohydrates content mg/g dry matter)											
100% NPK	128.10	143.11	102.22	101.17	120.09	**118.94**	164.10	198.12	103.10	100.11	112.19	**135.52**
100% NPK + BF	114.16	100.20	186.44	130.13	153.22	**136.83**	114.20	97.49	219.25	175.34	206.26	**162.51**
50% NPK + BF	131.29	95.09	188.07	106.29	123.31	**128.81**	185.06	101.00	228.21	103.32	142.15	**151.95**
BF	103.14	109.19	185.43	113.20	111.01	**124.39**	101.11	106.17	209.31	115.00	109.29	**128.18**
Means	**119.17**	**111.90**	**165.54**	**112.70**	**126.91**	---	**141.12**	**125.69**	**189.97**	**123.44**	**142.47**	---

*** 100% NPK = Ammonium sulphate at 400 kg/fed. + calcium superphosphate at 150 kg/fed. + potassium sulphate at 50 kg/fed.**
BF = Biofertilization using *Azotobacter chroococcum* as a preplanting seed treatment and post planting top dressings.

The results obtained with different combinations of pinching and fertilization treatments (in terms of the total carotenoids content) also differed from one season to the other (Table 5). In the first season, the highest value was obtained from plants pinched using kinetin at 15 ppm, and fertilized using 50% NPK + biofertilization. However, the highest value recorded in the second season was obtained from plants that received the same fertilization treatment (50% NPK + biofertilization), but were pinched manually.

b. Total carbohydrates content

The effect of pinching and fertilization on the content of total carbohydrates in dried leaves of *Hibiscus sabdariffa* L. plants is demonstrated by the data in Table (5). In both seasons, manual pinching decreased the content of total carbohydrates, compared to control plants. Chemical pinching using kinetin at 30 ppm also decreased the content of total carbohydrates in both seasons, compared to the control. On the other hand spraying kinetin at 15 or 45 ppm increased the content of total carbohydrates, compared to the control, with kinetin at 15 ppm giving the highest values in both seasons. A similar conclusion was obtained by Ibrahim and Tarraf (2000), who mentiond that spraying Egyptian fennel (*Foeniculum vulgar* Mill. var. *dulce*) plants with kinetin generally increased the total carbohydrates content.

Fertilization of roselle plants with combinations of NPK (at 100% or 50%) and biofertilization generally increased the total carbohydrates content in leaves of roselle plants, compared to the control. In both seasons, supplying roselle plants with 100% NPK + biofertilization gave the highest carbohydrates content, compared to values obtained from plants receiving any other fertilization treatment. On the other hand, supplying the plants with biofertilization alone gave results which varied from one season to the other. In the first season, using biofertilization alone increased the total carbohydrates content, compared to the control, but in the second season, an opposite result was recorded, i.e. biofertilization gave a lower value than the control.

The data in Table (5) also show the effect of different combinations of pinching and fertilization treatments on the total carbohydrates content. In both seasons, the highest values were obtained from plants pinched chemically using kinetin at 15 ppm, and fertilized using 50% NPK + biofertilization.

c. Nutrient contents

- Nitrogen

Data presented in Table (6) demonstrate the effect of manual and chemical pinching on the N content in leaves of roselle plants. In both seasons, pinching the plants manually, or chemically using kinetin at 15 ppm or 30 ppm, increased the content of nitrogen, compared to the control. On the other hand, plants sprayed with kinetin at 45 ppm had a lower N content than control plants (in both seasons). The most effective pinching treatment for increasing N uptake and accumulation in roselle leaves was spraying the plants with kinetin at 15 ppm.

Table (6): Effect of manual pinching, kinetin, NPK and biofertilization treatments on the contents of N, P and K in leaves of roselle (*Hibiscus sabdariffa*) plants in the 1999 and 2000 seasons.

*Fertilization treatments (B)	First season (1999)						Second season (2000)					
	Pinching methods (A)					Means	Pinching methods (A)					Means
	Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)		Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)	
	N content (% of dry matter)											
100% NPK	3.00	3.50	3.70	3.00	3.15	**3.27**	2.50	2.80	3.15	2.62	2.24	**2.66**
100% NPK + BF	3.20	3.65	3.85	3.46	3.13	**3.46**	2.55	2.95	3.27	2.63	2.03	**2.69**
50% NPK + BF	3.27	3.80	3.78	3.48	3.17	**3.50**	2.60	3.27	3.31	2.74	2.34	**2.85**
BF	2.80	2.20	2.91	2.59	2.62	**2.62**	2.10	2.31	2.45	2.38	2.41	**2.33**
Means	**3.07**	**3.29**	**3.56**	**3.13**	**3.02**	—	**2.44**	**2.83**	**3.04**	**2.59**	**2.25**	---
	P content (% of dry matter)											
100% NPK	0.095	0.150	0.200	0.170	0.137	**0.150**	0.110	0.125	0.230	0.130	0.100	**0.139**
100% NPK + BF	0.190	0.180	0.220	0.260	0.140	**0.198**	0.155	0.150	0.260	0.270	0.130	**0.193**
50% NPK + BF	0.070	0.200	0.220	0.210	0.190	**0.178**	0.096	0.170	0.240	0.250	0.172	**0.186**
BF	0.040	0.060	0.065	0.092	0.102	**0.072**	0.050	0.077	0.095	0.067	0.080	**0.074**
Means	**0.099**	**0.148**	**0.176**	**0.183**	**0.142**	—	**0.103**	**0.131**	**0.206**	**0.179**	**0.121**	---
	K content (% of dry matter)											
100% NPK	2.19	1.91	2.27	2.00	1.86	**2.05**	1.89	1.40	1.74	1.57	1.33	**1.59**
100% NPK + BF	2.15	2.46	2.32	2.42	2.21	**2.31**	1.79	2.29	1.98	2.27	1.90	**2.05**
50% NPK + BF	2.14	2.42	2.34	2.36	2.22	**2.30**	1.59	2.25	2.25	2.00	1.91	**2.00**
BF	1.85	2.15	2.13	2.05	2.11	**2.06**	1.31	1.61	1.53	1.46	1.34	**1.45**
Means	**2.08**	**2.24**	**2.27**	**2.21**	**2.10**	—	**1.65**	**1.89**	**1.88**	**1.83**	**1.62**	---

* 100% NPK = Ammonium sulphate at 400 kg/fed. + calcium superphosphate at 150 kg/fed. + potassium sulphate at 50 kg/fed.
BF = Biofertilization using *Azotobacter chroococcum* as a preplanting seed treatment and post planting top dressings.

Regarding the effect of fertilization, it is clear from the data in Table (6) that supplying roselle plants with combinations of chemical NPK fertilization and biofertilization (100% NPK + biofertilization, or 50% NPK+ biofertilization) increased the N content in both seasons, compared to the control. In both seasons, plants fertilized with 50% NPK+ biofertilization had higher N contents than those receiving any other fertilization treatments. The increase in the content of nitrogen may be attributed to the role of *Azotobacter* bacteria in fixing nitrogen through their high level of respiration (Burris, 1976). However, plants supplied with biofertilization alone had the lowest N contents. Data recorded in Table (6) also reveal the effect of the different combinations of pinching and fertilization treatments on the N content. In the first season, fertilization with 100% NPK + biofertilization, combined with chemical pinching using kinetin at 15 ppm, gave the highest N content, compared to any other treatment combination. However, the highest value recorded in the second season was obtained from plants sprayed with kinetin at 15 ppm, and fertilized with 50% NPK + biofertilization.

- Phosphorus

The results recorded in the two seasons (Table 6) show that the different pinching treatments had a considerable effect on the P content in leaves of roselle plants. Both manual and chemical pinching increased the content of phosphorus in both seasons, whereas the lowest values were obtained from control plants. In the first season spraying the roselle plants with kinetin at the concentration of 30 ppm gave the highest P content, but in the second season, the highest value was obtained from plants sprayed with kinetin at 15 ppm. It was also clear that manual pinching was generally less effective than kinetin at 15 Or 30 ppm, but was more effective than kinetin at 45 ppm, in terms of promoting P uptake and accumulation in roselle leaves.

The data in Table (6) also show that fertilization of roselle plants with combinations of NPK and biofertilization (100% NPK+ biofertilization or 50 % NPK + biofertilization) increased the P content, compared to plants fertilized with 100% NPK alone (control). In both seasons, the highest mean P contents were those of plants fertilized with 100 % NPK+ biofertilization. In contrast, plants supplied with biofertilization alone had lower P contents in both seasons, compared to those found in control plants.

It is also clear from the data in Table (6) that the best combination of treatments for increasing the uptake and accumulation of P in roselle leaves was pinching chemically using kinetin at 30 ppm, and fertilization using 100% NPK + biofertilization. This combination of treatments gave higher values in both seasons, compared to those obtained from plants receiving any other treatment combination.

- Potassium

Data presented in Table (6) demonstrate the effect of pinching and fertilization treatments on the K content in leaves of *Hibiscus sabdariffa* L. plants. It was noticed that plants which were manually pinched had higher K contents, compared to unpinched control plants. Using kinetin at 15 or 30 ppm also increased the K content in both seasons, compared to the control.

However, the K content was decreased steadily as the kinetin concentration was raised from 15 to 30 or 45 ppm. As a result, the highest kinetin concentration (45 ppm) slightly increased the K content in the first season only, but decreased it in the second season, compared to the control.

Fertilization of roselle plants with combinations of NPK (at 100% or 50%) and biofertilization increased the K content in both seasons, compared to the that of plants receiving NPK fertilization only (control). In both seasons, plants fertilized with 100% NPK + biofertilization had the highest mean K contents, compared to plants receiving any other fertilization treatment. On the other hand, supplying the plants with biofertilization alone gave different results in the two seasons; in the first season it gave a slightly higher K content than the control, but in the second season, it decreased the K content, compared to the control.

Data presented in Table (6) also reveal the effect of the interaction between fertilization treatments and pinching methods on the K content. It was noticed that the combination of manual pinching, and fertilization using 100% NPK + biofertilization gave the highest K content. On the other hand, the lowest K content was obtained in unpinched plants, which were supplied with biofertilization alone.

2. Sepals chemical characteristics

a. pH-values

Data recorded in the two seasons (Table 7) demonstrate the effect of pinching and fertilization treatments on pH-values in sepals of *Hibiscus sabdariffa* L. var. *sabdariffa*. It was noticed that plants pinched manually had slightly lower pH-values, compared to the control plants. Spraying kinetin at 15 ppm also decreased the pH-values, compared to those of control plants. However, raising the kinetin concentration from 15 to 30 or 45 ppm caused a gradual increase in the pH-values. Accordingly, plants sprayed with the highest kinetin concentration (45 ppm) gave the highest pH-values recorded in both seasons.

The results presented in Table (7) also show that, in most cases, plants receiving biofertilization (alone or combined with NPK at 100% or 50%) had a lower pH-value than that of plants fertilized with 100% NPK alone. The only exception to this general trend was recorded in the first season, with plants fertilized using 100% NPK + biofertilization giving a higher pH-value than those obtained from all other treatments. In the second season, the highest pH-value was obtained as a result of fertilization using 100% NPK alone.

It is also clear from the data in Table (7) that combining chemical pinching using kinetin at 45 ppm, with fertilization using 100% NPK, gave the highest pH-values, compared to plants receiving any other combination of pinching methods and fertilization treatments. On the other hand, the lowest pH-values were obtained from plants pinched chemically using kinetin at 15 ppm, and supplied with 100% NPK + biofertilization.

Table (7): Effect of manual pinching, kinetin, NPK and biofertilization treatments on the pH value, total acidity, total soluble solids content (T.S.S.) and anthocyanin content in sepals of roselle (*Hibiscus sabdariffa*) in the 1999 and 2000 seasons.

*Fertilization treatments (B)	First season (1999)						Second season (2000)					
	Pinching methods (A)					Means	Pinching methods (A)					Means
	Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)		Control	Manual	Kinetin (15 ppm)	Kinetin (30 ppm)	Kinetin (45 ppm)	
	pH- values											
100% NPK	3.59	2.98	3.87	4.00	4.67	**3.82**	3.24	3.14	3.32	3.35	4.00	**3.41**
100% NPK + BF	4.61	4.40	2.80	3.62	4.20	**3.93**	3.43	3.41	3.10	3.28	3.40	**3.32**
50% NPK + BF	3.45	3.20	3.63	2.87	3.16	**3.26**	3.22	3.20	3.29	3.11	3.18	**3.20**
BF	3.26	3.51	3.00	3.95	3.48	**3.44**	3.21	3.27	3.15	3.33	3.22	**3.24**
Means	**3.73**	**3.52**	**3.32**	**3.61**	**3.88**	---	**3.27**	**3.25**	**3.21**	**3.27**	**3.45**	---
	Total acidity (%)											
100% NPK	3.98	5.78	4.56	3.37	2.68	**4.07**	4.99	6.67	5.10	4.24	2.99	**4.80**
100% NPK + BF	2.83	3.31	7.81	4.93	3.62	**4.50**	3.91	4.00	8.60	5.86	4.30	**5.33**
50% NPK + BF	5.06	5.09	4.40	6.62	5.87	**5.41**	5.99	6.58	5.00	7.01	6.76	**6.27**
BF	5.31	4.50	6.24	3.31	3.87	**4.65**	6.65	5.02	6.99	4.28	4.41	**5.47**
Means	**4.29**	**4.67**	**5.75**	**4.56**	**4.01**	---	**5.38**	**5.57**	**6.42**	**5.35**	**4.61**	---
	T. S. S. (%)											
100% NPK	3.40	3.00	3.60	2.00	2.80	**2.96**	4.20	3.00	4.00	2.30	3.60	**3.42**
100% NPK + BF	3.75	2.10	2.70	2.10	4.40	**3.01**	4.40	2.40	3.40	3.80	4.50	**3.70**
50% NPK + BF	3.50	2.90	4.80	4.60	3.70	**3.90**	4.10	3.60	5.80	5.70	3.50	**4.54**
BF	3.10	3.00	2.30	2.40	4.00	**2.96**	2.70	2.80	2.40	2.50	4.40	**2.96**
Means	**3.44**	**2.75**	**3.35**	**2.77**	**3.72**	---	**3.85**	**2.95**	**3.90**	**3.58**	**4.00**	---
	Anthocyanin content (mg / g dry matter)											
100% NPK	11.21	12.24	8.60	8.36	9.92	**10.07**	13.69	13.72	9.35	9.03	10.12	**11.18**
100% NPK + BF	9.18	9.08	16.98	12.95	9.89	**11.62**	9.84	10.00	20.30	18.94	12.98	**14.41**
50% NPK + BF	8.79	8.99	13.00	12.67	9.00	**10.49**	8.12	9.45	18.97	17.59	9.62	**12.75**
BF	7.32	8.04	12.52	6.99	6.77	**8.33**	6.08	7.20	13.82	9.09	6.17	**8.47**
Means	**9.12**	**9.59**	**12.77**	**10.24**	**8.89**	---	**9.43**	**10.09**	**15.61**	**13.66**	**9.72**	---

*** 100% NPK = Ammonium sulphate at 400 kg/fed. + calcium superphosphate at 150 kg/fed. + potassium sulphate at 50 kg/fed.**
BF = Biofertilization using *Azotobacter chroococcum* as a preplanting seed treatment and post planting top dressings.

b. Total acidity

The data in Table (7) show the total acidity percentages in sepals of roselle plants, as affected by the different pinching and fertilization treatments. In both seasons, manual pinching increased the total acidity, compared to that of control plants. Also, the total acidity percentage was increased by spraying kinetin at 15 ppm, which gave the highest average values, compared to values recorded with any other pinching treatment. Raising the kinetin concentration from 15 ppm to 30 or 45 ppm caused a steady reduction in the total acidity percentage. Accordingly, the lowest values recorded in the two seasons were obtained when the highest kinetin concentration (45 ppm) was used.

Concerning the effect of fertilization on the total acidity percentage, it was noticed that supplying roselle plants with biofertilization, with or without chemical NPK fertilization (at 100% or 50%), increased the total acidity percentage, compared to that of plants fertilized with 100% NPK alone. In both seasons, the most effective fertilization treatment for increasing the total acidity percentage was supplying the plants with 50% NPK + biofertilization. This treatment gave the highest mean values, compared to any other fertilization treatment.

Regarding the interaction between the effects of the pinching and fertilization treatments, it was noticed that plants sprayed with kinetin at 15 ppm and fertilized with 100% NPK + biofertilization had the highest total acidity percentages, compared to plants receiving any other combination of pinching and fertilization treatments. On the other hand, the lowest total acidity percentages were obtained from plants fertilized with 100% NPK only, and sprayed with kinetin at 45 ppm.

c. Total soluble solids (T.S.S.) content

The effect of the different pinching methods on the total soluble solids content in roselle sepals is represented by the data in Table (7). It can be stated that manual pinching decreased the total soluble solids content, compared to unpinched control plants. Spraying roselle plants with kinetin at 30 ppm also decreased the total soluble solids content, compared to the control. On the other hand, the highest mean values recorded in both seasons were obtained from plants sprayed with kinetin at 45 ppm. Spraying roselle plants with the lowest kinetin concentration (15 ppm) decreased the total soluble solids content in the first season, but increased it in the second season, compared to the control.

Fertilization of roselle plants with 100% NPK + biofertilization, or 50% NPK + biofertilization increased the total soluble solids in both seasons, compared to fertilization using 100% NPK alone. In both seasons, the highest mean values were obtained from plants fertilized with 50% NPK + biofertilization. These results are in agreement with the findings of Mahmoud and Amara (2000), who studied the effect of NPK, alone or combined with biofertilization, on tomato. They reported that biofertilization with *Bacillus subtilis* and *B. megatherium* (*B. megaterium*), combined with 50% of the recommended NPK dose, led to an increase in the T.S.S. content. On the other hand, supplying the plants with biofertilization only gave values which were equal to, or less than those obtained from control plants.

The data in Table (7) also show the values recorded for the total soluble solids, as affected by the interaction between the effects of pinching and fertilization treatments. In both seasons, combining chemical pinching using kinetin at 15 ppm, with fertilization using 50% NPK + biofertilization gave the highest total soluble solids contents, compared to values obtained from plants receiving any other combination of pinching and fertilization treatments.

d. Anthocyanin content

The data presented in Table (7) demonstrate the effect of pinching and fertilization treatments on the content of anthocyanin in dried roselle sepals. It was noticed that manual pinching increased the content of anthocyanin, compared to the control. This conclusion is in agreement with the findings of Omer *et al.* (1997) who stated that pinching of *Hibiscus sabdariffa* (Sabahia-17 light colour and Sabahia-17 dark colour) increased the anthocyanin yield in both cultivars. However, spraying kinetin at 15 or 30 ppm was generally more effective that manual pinching for increasing the anthocyanin content in roselle sepals. In both seasons, the highest anthocyanin content was detected as a result of treating roselle plants with kinetin at 15 ppm. On the other hand, raising the kinetin concentration from 15 ppm to 30 or 45 ppm resulted in a gradual reduction in the anthocyanin content. As a result, plants sprayed with the highest kinetin concentration (45 ppm) had a lower anthocyanin content in the first season than that of unpinched control plants.

Fertilization of roselle plants using combinations of NPK (at 100% or 50%) and biofertilization increased the anthocyanin content, compared to plants fertilized with 100% NPK alone. In both seasons, plants fertilized with 100% NPK + biofertilization had the highest anthocyanin content in their sepals, compared to plants receiving any other fertilization treatment. On the other hand, supplying roselle plants with biofertilization alone had an adverse effect on the synthesis and accumulation of anthocyanin in the sepals. In both seasons, sepals of plants supplied with biofertilization only, had the lowest anthocyanin contents.

The interaction between the effects of the tested pinching and fertilization treatments on the anthocyanin content is shown in by the data in Table (7). By comparing the values obtained from plants receiving the different combinations of pinching methods and fertilization treatments, it was noticed that in both seasons, plants pinched chemically using kinetin at 15 ppm, and fertilized using 100% NPK + biofertilization, had the highest anthocyanin content in their sepals, compared to plants receiving any other combination of pinching and fertilization treatments.

Recommendations: From the above results, it can be recommended that roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*) plants should be sprayed with kinetin at 15 ppm, and fertilized using 50% NPK + biofertilization. This combination of treatments gave the best results, in terms of number of branches and fruits/plant, fresh and dry weights of sepals/plant, the total carbohydrates content in leaves, as well as the content of total soluble solids in the sepals, compared to any other combination of pinching and fertilization treatments.

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تأثير معاملات التطويش و التسميد على النمو الخضرى و إنتاج السبلات والتركيب الكيميائى فى الكركديه (*Hibiscus sabdariffa* L.)

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أجريت هذه الدراسة فى مشتل التجارب بقسم بساتين الزينة، كلية الزراعة، جامعة القاهرة بالجيزة، و ذلك خلال الموسمين المتتاليين ١٩٩٩ و ٢٠٠٠، بغرض دراسة تأثير التطويش اليدوى والكيميائى (بإستخدام الرش الورقى بالكينيتين) و كذلك معاملات التسميد الكيماوى (NPK) والحيوى على النمو و إنتاج السبلات و التركيب الكيميائى لنباتات الكركديه(Roselle, *Hibiscus sabdariffa* L. var. *sabdariffa*, cv. Sabahia 17).

عوملت نباتات الكركديه بإحدى المعاملات التالية: (١) تركت بدون تطويش ، (٢) تم تطويشها يدويا (مرتان، الأولى بعد شهر من الزراعة، و الثانية بعد الأولى بشهر) أو (٣) تم تطويشها كيميائياً عن طريق رش الأوراق بالكينيتين بتركيزات ١٥، ٣٠، ٤٥ جزء/المليون (أجريت المعاملة ثلاث مرات على فترات ١٥ يوم، إبتداءً من بعد الزراعة بثلاثين يوم). كذلك تم إمداد النباتات بالتسميد الكيماوى NPK [بإستخدام سلفات الأمونيوم (٢٠,٥ % نتروجين) بمعدل ٤٠٠ كيلو جرام/ فدان، و سوبر فوسفات الكالسيوم (١٥,٥ % فو٢أ٥) بمعدل ١٥٠ كيلو جرام/ فدان، و سلفات البوتاسيوم (٤٨ % بو٢أ) بمعدل ٥٠ كيلو جرام/ فدان] أو التسميد الحيوى (بمفرده أو بالإشتراك مع التسميد الكيماوى بإستخدام ٥٠% أو ١٠٠% من المعدلات السابقة). و قد إستخدمت النباتات المسمدة بواسطة ١٠٠% NPK بدون تسميد حيوى كنباتات مقارنة (كنترول). و فى المعاملات التى تضمنت إستخدام التسميد الحيوى، تم خلط السماد الحيوى (وهو خليط من الفيرميكوليت و بكتيريا *Azotobacter chroococcum* بتركيز ١٠^٩ بكتريا/ ١ جم فيرميكوليت) بالبذور قبل الزراعة، و ذلك بمعدل ٥٠٠ جم من السماد الحيوى / ٢٥٠ جم بذور. كما أضيف التسميد الحيوى فى صورة سائلة للتربة بعد الزراعة، و ذلك ثلاث مرات فى كل موسم (على فترات ٣٠ يوم) و كان السائل المضاف هو خليط من المولاس و بكتيريا *Azotobacter chroococcum* .

من بين معاملات التطويش اليدوى و الكيميائى المختلفة، فإن المعاملة بالكينيتين بتركيز ١٥ جزء فى المليون أعطت أعلى القيم لمعظم صفات النمو و المحصول التى تم دراستها (إرتفاع النبات، عدد الأفرع و الأوراق و الثمار/نبات، الأوزان الطازجة و الجافة للأوراق و السبلات/نبات). كما أدت المعاملة بالكينيتين إلى زيادة محتوى الكلوروفيلات الكلية و الكاروتينويدات والكربوهيدرات الكلية و النتروجين و الفوسفور و البوتاسيوم فى الأوراق، و كذلك محتوى الحموضة الكلية و الأنثوسيانين فى السبلات. هذا و أعطى التسميد بإستخدام ١٠٠% NPK + التسميد الحيوى أعلى القيم لصفات عدد الأفرع و الأوراق و الثمار/ نبات، و الأوزان الطازجة و الجافة للأوراق/نبات، ومحتوى الكربوهيدرات الكلية و الفوسفور و البوتاسيوم فى الأوراق، و كذلك محتوى الأنثوسيانين فى السبلات، و هذا مقارنة بجميع معاملات التسميد الأخرى. إلا أن أعلى أوزان طازجة و جافة للسبلات تم الحصول عليها عند التسميد بإستخدام ٥٠% NPK + التسميد الحيوى ، كما أعطت هذه المعاملة زيادة غير معنوية فى عدد الأفرع/نبات، مقارنة بالأفرع المتكونة على النباتات المسمدة بإستخدام ١٠٠% NPK فقط، و تدل هذه النتائج على أن التسميد الحيوى قلل من الإحتياج للأسمدة الكيماوية (اللازمة للتفريع و إنتاج السبلات) بمقدار لا يقل عن ٥٠% . كذلك فإن التسميد بإستخدام ٥٠% NPK + التسميد الحيوى أعطى أعلى محتوى من الكوروفيلات الكلية و النتروجين فى الأوراق، و أعلى محتوى من الحموضة الكلية و المواد الصلبة الذائبة فى السبلات. و بالإضافة إلى ما سبق فإن الجمع بين المعاملة بالكينيتين بتركيز ١٥ جزء فى المليون، و التسميد بإستخدام ٥٠% NPK + التسميد الحيوى أدى إلى إعطاء أفضل النتائج من حيث عدد الأفرع و الثمار/نبات، و الأوزان الطازجة و الجافة للسبلات/نبات، و محتوى الكربوهيدرات الكلية فى الأوراق، و كذلك محتوى المواد الصلبة الذائبة الكلية فى السبلات، و هذا بالمقارنة بأى توليفة أخرى من معاملات التطويش و التسميد.