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NITROGEN FERTILIZER REQUIREMENTS OF *STEVIA REBAUDIANA BERTONI,* UNDER EGYPTIAN CONDITIONS

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Abstract

Field trail was carried out during 1998 to 2000 at Giza Experimental Station (ARC), to find out the suitable nitrogen rate and cutting time that would insure optimum stevia yields and stevioside content. Three nitrogen rates (10, 20 and 30 Kg N/F) and ten cutting times (from July 1998 at a two month interval up to Feb. 2000). Results showed a gradual and significant increase in fresh and dry leaves, stem, biomass yields, total soluble carbohydrate and stevioside, over and within cuts as nitrogen fertilizer increased up to 30 Kg N/F.

Dry leaves yield and stevioside content (represent stevia economic yield and quality) increased by 64% and 1.99% at 30Kg N/F as compared to 10 Kg N/F.

All studied traits showed significant increase in all successive cuts, but they manifested a remarkable increase during the summer cuts and surpassed those during winter cuts.

These results give evidence that climate conditions such as temperature, length and intensity of photoperiod greatly affected stevia economic production and quality parameters more than successive cuts .

The interaction between nitrogen fertilizer levels and cutting time significantly affected most studied traits. The highest dry leaves yield/F and leaves stevioside content were obtained from summer cuts fertilized with 30 Kg N/F.

INTRODUCTION

Reduction of the extremely high sugar consumption and its substitution with natural sweeteners are quite important problems, especially artificial substances used in present day food industry such as saccharine, sorbine, aspartame. Hence, they have not always met the producers and consumers requests.

The food value of the plant *Stevia rebaudiana* Bertoni is given by the presence in its complex structure of diterpenoid glycosides group, which confers a high sweet degree. The sweet component of the plant is 300 times sweeter than sucrose and has similar tasteful properties. The hypocaloric sweetener of Stevia arouses interest also because it is a natural product. The main sweet substance called stevioside has been already used currently in many countries such as Paragway, Japan, Brazil, California, China and others in order to sweeten a variety of foods. Therefore, maximize stevia productivity in terms of leaves yield and sweet substances depending on the growing temperature and nutritious elements supply. It is well known that nitrogen had a vital role on the growth and physiological processes of any plant. Numerous investigations discussed the effect of nitrogen fertilizer on yields and quality of stevia (Angkapradipta *et al.* 1986; Kornieko *et al.* 1995; Lima- Filho *et al.* 1997 and Utumi *et al.* 1999). Also harvest of successive cuts received attentions.

The objective of this study was to find out the suitable nitrogen rate and cutting time that would insure optimum stevia leaves yield and stivioside content.

MATERIALS AND METHODS

Seeds of stevia variety Spanti imported from Spain was planted in glass house on Feb. 15, 1998 in plastic pots (15 cm diameter x 25 cm height) filled with a mixture of sand and silt clay soil (1:1w/w). After 7 days from sowing and periodically every 10 days intervals seedlings were sprayed with a nutritive solution (1.5 g Zn, 0.4 g Cu, 1.2 g Fe, 1.2 g B and 0.2 g Mo/20L water).

On April 18, 1998 seedlings at two month age, were transplanted to the permanent experiment site in Giza Experimental Station, Agricultural Research Centre (ARC).

Factorial experiment in randomized complete block design with four replications was used. Three nitrogen levels, i.e. 10, 20 and 30 Kg N/F were added after each cutting, and ten successive cuttings were carried out on the following dates.

1- 21/7/1998	2- 20/9/1998	3- 20/11/1998
4- 20/2/1999	5- 20/5/1999	6- 20/7/1999
7- 20/9/1999	8- 20/11/1999	9- 20/2/2000
10- 20/5/2000		

Plot size was $21m^2$ (5 ridges 6 m long and 70 cm apart), spacing between hills were 15 cm to give target plant population of 40000/F. For each cutting, nitrogen fertilizer was added as Urea (46% N) in two equal doses after cutting and after 15 days. Moreover, 15 Kg P_2O_5 in the form of super phosphate 15% P_2O_5 per feddan was added in single dose at land preparation, other cultural practices such as hoeing, irrigation ...etc. were maintained at levels to assure optimum production. Cutting was carried out at 3-5 cm above soil surface on above mentioned dates.

The three middle ridges were used to determine fresh and dry leaves, stem and biomass yield, leaves and stem were dried in shade under natural condition. To determine total soluble carbohydrates, leaves samples were taken from each cut and replicate and dried in an electric oven with air draft at 70°c, total soluble carbohydrates was determined according to AOAC (1990) method, moreover, stevioside was calculated according to Nishiyama *et al.* (1991) formula:

Tc = 7.56+0.96 St where,

Tc = total soluble carbohydrates and St= steviosoide content.

The soil was analyzed according to Chapman and Pratt (1961) and the results are given in (Table 1) .

Table 1. The properties of the experimental soil

Chemical properties

30 cm (depth) Ec (mmhos/cm)1.85

Macro and micro elements (ppm)

N	P	K	Ca	F	Zn	Mn	Cu
2.07	2.67	171.60	146.20	12.30	1.12	1.08	3.95

Anions (meq/L)

HCO_3^-	CL^-	SO_4^-
2.03	6.65	9.94

Cations (meq/L)

Ca^{++}	Mg^{++}	Na^+	K^+
4.95	4.95	8.05	0.23

Physical Properties

Clay	Silt	Fine sand	Coarse sand
35.80	38.26	24.14	1.80

RESULTS AND DISCUSSION

Soil properties of the experimental sites as reported in (Table 1) show that the soil is silty clay textured, free from salinity hazard and contains adequate concentration of most macro and micro elements except nitrogen and phosphorus. Irrigation is carried out by surface methods using ground water with acceptable quality.

Fresh and dry yield of stevia leaves

Data in (Table 2 and 3) show the effect of nitrogen dressing on the productivity of fresh and dry leaves of stevia. Linear and significant increase in fresh and dry leaves was associated with the increase in nitrogen rates. The percent of increase in fresh leaves yield amounted 10.4 (from 4.23 to 4.67 T/F) and 19.15 (from 4.23 to 5.04 T/F) as nitrogen fertilizer rates increased to reach 20 and 30 Kg/F as compared with 10 Kg N/F (Table 2). Keeping the same trend of improvement for leaves dry yield has been also observed with increasing the rate of N application, the percent of increase in dry leaves yield recorded 38.18 (from 1.10 to 1.52 T/F) and 63.64 (from 1.10 to 1.80 T/F) corresponding to 20 and 30 Kg N/F as compared with 10 Kg N/F (Table 3 and fig 1). These results give evidence that the soil was initially incapable of supplying the crop with its N requirement, in addition stevia under the condition of this work may give more response to increasing N dressing over 30 Kg N/F.The obtained results are in line with those of Angkapradipta *et al.* (1986) who reported that nitrogen fertilizer significantly increased stevia foliage fresh and dry matter production. Lima - Filho *et al.* (1997, a) stated that in Brazil, the production of one ton of dry leaves of *S. rebaudina* required 64.6 Kg N.

Regarding the effect of cutting times on fresh and dry leaves yield/F (Tables 2 and 3), averaged data showed significant differences among cuts. The highest fresh and dry leaves yield were of summer cuts (May, July and Sept.) while winter cuts which carried out in this study during Feb. and Nov., fresh and dry leaves yield were markedly (statistically significant) decreased. Such effect may be due to the fact that stevia growth is greatly favored by high temperature, light and moisture. Richard (1996) reported that stevia grows naturally in a semi-humid subtropical climate with a temperature range of 21°F to 110°F (an average temperature of 75°F). In low temperature and short days stevia plants tend to flower and set seeds. In this connection Healy and Graper (1989) noticed that short days are required to promote stevia flowering and 16h photoperiod maintained the plants in vegetative condition. Data (Table 3) also cleared that the percent of increase in fresh leave yield recorded 16.2, 50.1, 74.0 and 72.4 in May, 1999 (5th cut) July 1999 (6th cut), Sept., 1999 (7th cut) and May, 2000 (10th cut), respectively as compared with the first cut (July 1998) (Table 2), while the corresponding dry leaves yield for the same cuts were 16.26, 49.59, 60.16 and 69.92 respectively (Table 3).

Table 2. Fersh yield of stevia as affected by nitrogen fertilizer and cutting time

Cutting time (C)	Leave yield (t/f) N rates (kg/f)				Relative changes	Stem yield (t/f) N rates (kg/f)				Relative changes	Biomass yield (t/f) N rates (kg/f)				Relative changes
	10	20	30	Average		10	20	30	Average		10	20	30	Average	
21.7.1998	3.30	3.79	4.24	3.77	100.0	6.21	6 88	7.40	6.84	100.00	9.54	10.67	11.64	10 61	100.00
20.9.1998	3.57	4.43	4.73	4.25	112.7	7.15	705.00	7.49	723.00	105.70	10.72	11.48	12.22	1147.00	108.11
20.11.1998	2.72	3.04	3.43	3.06	81.2	6.27	7.00	7.65	6.97	101.90	8.99	10.04	11.08	10.04	94.63
20.2.1999	2.57	2.75	3.15	2.82	74.8	5.60	6.25	7.08	6.31	92.25	8.17	9.00	10.23	9.13	86.05
20.5.1999	3.96	4.49	4.69	4.38	116.2	7.15	7.71	8.01	7.72	112.87	11.41	12.20	12.70	12.10	114.04
20.7.1999	5.19	5.67	6.10	5.66	150.1	7.65	7.77	7.91	7.77	113.60	12.84	13.14	14.01	13.43	126.58
20.9.1999	6.13	6.62	6.92	6.56	174.0	8.31	8.58	9.47	8.79	128.51	14.44	15.20	16.39	15.34	164.58
20.11.1999	4.24	4.66	4.94	4.61	122.3	8.10	9.02	9.71	8.94	130.70	12.30	13.68	14.65	13.56	127.80
20.2.2000	4.48	4.89	5.38	4.91	130.2	8.32	8.68	9.16	8.72	127.49	12.80	13.57	14.54	13.64	128.56
20.5.2000	6.15	6.54	6.82	6.50	172.4	8.81	9.86	11.20	10.00	146.20	11.96	16.40	18.02	16.46	155.14
Average	4.23	4.67	5.04	4.65		7.39	7.88	8.52	7.93		11.62	12.55	13.56	12.58	
Relative changes	100.0	110.40	119.15			100.0	106.63	115.29			100.0	108.0	116.70		

L. S. D. 5%	Leave yield	Stem yield	Biomass yield
N	0.12	0.29	0.26
C	0.08	0.13	0.11
N x C	N. S.	0.42	0.35

Furthermore, fresh and dry leaves yield were increased gradually latest in successive cuts in both winter and summer and this effect was more pronounced in later cuts than early ones. Such effect is due to the augmentation of basal buds, new tillers and branches that developed with subsequent cutting . These results are in line with those of Shyu *et al.* (1994) who reported that harvesting date had a significant effect on tiller number and fresh dry weight of leaves.

Fresh and dry yield of stem

A gradual and significant increase in fresh and dry yield of stem (including main stem, tillers and lateral branches) was apparent as nitrogen dressing increased to reach its maximum values recording (15.29% and 63.16% for fresh and dry stem yield, respectively) corresponding to 30Kg N/F, as compared with 10Kg N/F (Tables 2 and 3). These results give evidence to the positive effect of nitrogen dressing on the growth of new tillers and lateral branches and hence leaves which reflected on the yield of fresh leave as mentioned before.

Significant differences in fresh and dry stem yield/F of leaves have been detected among successive cuts (Table 2 and 3). Data also manifested that fresh and dry stem yield showed the same trend as mentioned in leaves yield, the highest fresh and dry stem yield were in summer cuts, however, the lowest ones were of winter cuts (Table 2 and 3). Moreover, both traits were increased gradually in the successive cuts. Such effect may be due to the stimulatory effect of high temperature and frequency of cutting . Similar results were reviewed by Shyu *et al.* (1994) who stated that harvesting date had significant effect on fresh and dry weight of stem.

Fresh and dry biomass yield

Fresh and dry biomass yield significantly responded to nitrogen fertilizer (Tables 2 and 3). where a gradual increase in both traits have been detected as nitrogen rate increased up to 30Kg N/F compared with 10 Kg N/F. The percent of increase in fresh biomass averaged 8.00 and 16.70 on the application of 20 and 30 Kg N/F, respectively (Table 2). The corresponding percent of increase in dry biomass yield were 36.63 and 63.38 (Table 3). The increase in biomass yields may be due to the positive effect of nitrogen fertilizer on leaves and stem yields as mentioned before. In this connection Angkapradipta *et al.* (1986) showed that N fertilizer did not significantly increase the biomass production. Kornieko *et al.* (1995) reported that 80Kg N+ 80Kg P_2O_5 and 80Kg K_2O/ha was producing the highest fresh and dry biomass. Furthermore, Lima-Filho *et al.* (1997, b) and Utumi *et al.* (1999) showed that N deficiency results in re-

Table 3. Dry yield of Stevia as affected by nitrogen fertilizer and cutting time .

Cutting time (C)	Leave yield (t/f) N rates (kg/f) 10	20	30	Average	Relative changes	Stem yield (t/f) N rates (kg/f) 10	20	30	Average	Relative changes	Biomass yield (t/f) N rates (kg/f) 10	20	30	Average	Relative changes
21.7.1998	0.90	1.24	1.53	1.23	100.0	1.18	1.62	1.93	1.58	100.0	2.08	2.86	3.46	2.80	100.00
20.9.1998	0.99	1.47	1.72	1.39	113.01	1.31	1.64	1.98	1.65	104.43	2.30	3.11	3.70	3.04	108.57
20.11.1998	0.70	0.95	1.20	0.95	77.24	1.07	1.43	1.80	1.43	90.51	1.77	2.38	3.00	2.38	85.00
20.2.1999	0.66	0.86	1.10	0.87	70.73	0.90	1.26	1.80	1.32	83.54	1.56	2.12	2.90	2.19	78.21
20.5.1999	1.09	1.49	1.70	1.43	116.26	1.44	1.94	2.17	1.85	117.09	2.53	3.43	3.87	3.28	117.14
20.7.1999	1.42	1.87	2.21	1.84	149.59	1.47	1.88	2.15	1.84	116.46	2.89	3.75	4.36	3.67	131.07
20.9.1999	1.56	2.03	2.33	1.97	160.16	1.56	2.15	2.52	2.08	131.65	3.12	4.18	4.85	4.05	144.64
20.11.1999	1 09	1.47	1.75	1.44	117.07	1.34	1.86	2.19	1.80	113.92	2.43	3.33	3.94	3.23	115.36
20.2.2000	1.13	1.59	1.89	1.54	125.20	1.43	1.82	2.33	1.86	117.72	2.56	3.41	4.22	3.40	121.43
20.5.2000	1.45	2.27	2.54	2.09	169.92	1.57	2.31	2.87	2.25	142.41	3.02	4.58	5.41	4.34	155.00
Average	1.10	1.52	1.80	1.48		1.33	1.79	2.17	1.77		2.43	3.32	3.97	3.24	
Relative changes	100.0	138.18	163.64			100.0	134.59	163.16			100.0	136.63	163.38		
L. S. D. 5%															
N			0.09					0.03					0.11		
C			0.04					0.01					0.06		
N x C			0.13					0.04					0.19		

Table 4. Total soluble carbohydrate and stevioside content of Stevia leaves as affected by nitrogen fertilizer and cutting time (on dry matter basis)

Cutting time (C)	Total soluble carbohydrates (%) N rates (kg/f)				Relative changes	Stevioside (%) N rates (kg/f)				Relative changes
	10	20	30	Average		10	20	30	Average	
21.7.1998	50.80	51.15	51.40	51.12	100.0	45.04	45.41	45.67	45.37	100.0
20.9.1998	51.03	51.40	51.71	51.38	100.50	45.29	45.67	45.99	45.65	100.60
20.11.1998	49.88	50.27	50.56	50.24	98.28	44.09	44.49	44.79	44.46	98.00
20.2.1999	48.85	49.47	50.13	49.48	96.79	43.01	43.66	44.34	43.67	96.25
20.5.1999	50.51	50.95	51.03	50.83	99.43	44.74	45.20	45.28	45.07	99.34
20.7.1999	51.66	52.15	52.57	52.13	102.00	45.94	46.45	46.89	46.43	102.30
20.9.1999	51.57	52.30	53.08	52.32	102.40	45.85	46.61	47.41	46.62	102.80
20.11.1999	50.19	50.50	51.15	50.61	99.00	44.41	44.72	45.41	44.85	98.85
20.2.2000	49.89	50.33	50.54	50.26	98.32	44.10	44.56	44.78	44.48	98.04
20.5.2000	50.86	51.44	51.70	51.33	100.40	45.11	45.71	45.98	45.60	100.50
Average	50.52	51.00	51.39	50.95		44.76	45.19	45.65	45.22	
Relative changes	100.0	100.95	101.72			100.0	100.96	101.99		

L. S. D. 5%	Total soluble carbohydrates	Stevioside
N	0.36	0.37
C	0.11	0.11
N x C	0.34	0.35

Fig.1 Effect of nitrogen fertilizer on stevia fresh and dry leave yield

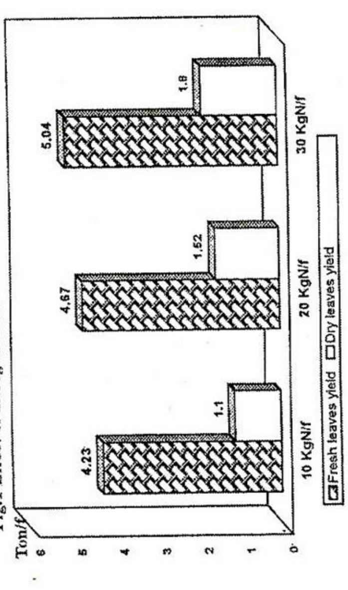
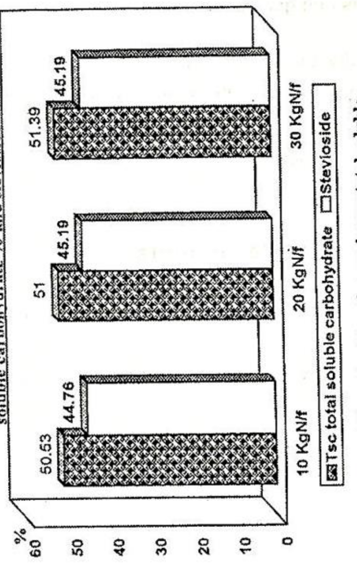
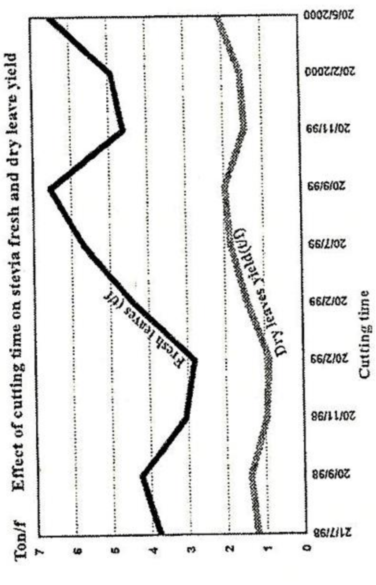


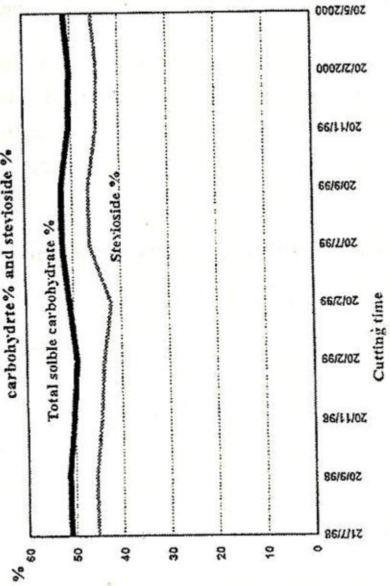
Fig.2 Effect of nitrogen fertilizer on leave total soluble carbohydrate % and stevioside %



Effect of cutting time on stevia fresh and dry leave yield



Effect of cutting time on leaves total soluble carbohydrte% and stevioside %



duced development, small leave few branches and generalized yellowing.

Significant differences in fresh and dry biomass yield among successive cuts have been detected (Tables 2 and 3). Biomass yield is the sum product of leave and stem, therefore, these traits (fresh and dry yield) have the same trend as in leaves and stem yields. The highest fresh and dry biomass yield were recognized in summer cuts and in latest cuts as well (Tables 2 and 3).

Total soluble carbohydrate and steviosoide contents

Total soluble carbohydrate is a good indicator for stevioside content in stevia leaves, where a positive correlation was found between both as reported by Nishiyama *et al.* (1991). Stevioside is considered the main sweet substance and has similar sweetening taste as sugar (sucrose) but its sweetness is 300 times as compared to sucrose (Buddhasukh and Vaneesorn, 1990, Wongrattanasathit, 1990 and Richard, 1996).

Data (Table 4) show a gradual and significant increase in total soluble carbohydrates and stevioside contents as nitrogen dressing increased. The percent of increase for total soluble carbohydrates were 0.95 and 1.72 and for stevioside 0.96 and 1.99 corresponding to nitrogen application of 20 and 30 Kg/F as compared to 10Kg N/F (Table 4, Fig 2). Such effect may be due to the stimulatory effect of N dressing on photosynthetic rate which reflect on better growth and quality. In this connection Utumi *et al.* (1999) reported that N deficiency decreased the stevioside content per plant.

With respect to the effect of cutting times on leaves contents of total soluble carbohydrates and stevioside (Table 4). Averaged values of both traits were significantly differed among successive cuts. The highest total soluble carbohydrates and stevioside were of summer cuts (July and Sept.,1999), while, winter cuts showed a *vise versa* trend. Similarly, Shyu *et al.* (1994) reported that harvest time significantly affected leaves content of sweet components.

Data (Table 4) obviously showed that temperature had a great effect on leaves content of total soluble carbohydrates and stevioside than frequency of cutting does. The first and second cuts (July and Sept., 1998) had a higher values of both traits than the 8th and 9th cuts which carried out during winter months (Nov.,1999 and Feb., 2000). Such effect is due to the fact that stevia tends to flower during short cold days and this may have a great implicate on the diminution of total soluble carbohydrates and stevioside contents.

Interaction between nitrogen dressing and cutting time significantly affected all studied traits except fresh leave yield in tons/F (table 2, 3 and 4). The highest leaves dry yield and stevioside were obtained from summer cuts fertilized with 30 Kg N/F.

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إحتياجات الإستيفيا من السماد الأزوتي تحت الظروف المصرية

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أجريت هذه الدراسة بمحطة البحوث الزراعية بالجيزة مركز البحوث الزراعية خلال الفترة من ١٩٩٨ حتى ٢٠٠٠ بهدف تحديد المعدل المناسب للسماد الآزوتي ومواعيد الحش لتحقيق أعلى إنتاجية من المحصول والنسبة المئوية لمركب الاستيوفيوسيد في أوراق الأستيفيا. وقد أستخدم في ذلك ثلاث معدلات من السماد الآزوتي (١٠ و٢٠ و٣٠ كجم نيتروجين/فدان) وتضاف بعد كل حشة وعدد عشر حشات (بداية من يولية ١٩٩٨ وكل شهرين حتى فبراير ٢٠٠٠) وقد أوضحت نتائج تحليل التباين ما يلي:

زيادة تدريجية معنوية في المحصول الغض والجاف للأوراق والسيقان والمحصول الكلي وكذا في الكربوهيدات الذائبة الكلية والإستيفيوسيد في كل حشة وكمتوسط عام لجميع الحشات بزيادة التسميد الآزوتي حتى ٣٠ كجم/ الفدان حيث بلغت الزيادة في محصول الأوراق الجاف ومركب الإستيفيوسيد ٦٤٪ و٩٩,١٪ على التوالي مقارنة بإضافة ١٠ كجم نيتروجين/فدان للحشة الواحدة.

حدثت زيادة معنوية في جميع الصفات تحت الدراسة بتوالي عملية الحش وهذه الزيادة كانت أكثر وضوحاً في الحشات الصيفية (حشات مايو – يوليو – سبتمبر). أوضحت النتائج أن تأثير درجات الحرارة وطول وشدة الإضاءة (حشات صيفية) كان أكثر وضوحاً على محصول الأوراق الجافة ومكون الإستيفيوسيد عن تكرار عمليات الحش. وكان أثر التفاعل بين معدلات التسميد الآزوتي ومواعيد الحش معنوياً على معظم الصفات وأن أعلى محصول من الأوراق الجافة للفدان وأعلى نسبة أستيفيوسيد في الأوراق نتجت من الحشات الصيفية والتي تم تسميدها بـ ٣٠ كجم آزوت /الفدان/ للحشة الواحدة.