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INTEGRATED EFFECT OF MAGNETIZED WATER AND BIOFERTILIZERS FOR ENHANCING THE PRODUCTIVITY OF *MAJORANA HORTENSIS* L. PLANT

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ABSTRACT: This investigation was carried out at the experimental farm of the faculty of Agriculture, Menoufia Univ. at Shiben El-Kom during to growing the seasons of 2017 and 2018 to study the effect of some magnetized water and biofertilization on the growth, herb yield and essential oil content of *Majorana hortensis* L. plants. The N_2-fixing bacterial strains of *Rizobium, Azospirillium* and *Azotobacter* were used as biofertilizers in current study. The plants were irrigated either with normal or magnetized irrigation water. The magnetized water was obtained by passing the water through magnetron tube of 2 inches diameter and 4000 Gauss's strains. The obtained results showed that the growth and herb yield parameters were markedly improved as a result of watering with magnetic water besides using bio fertilizers (*Rhizobium, Azotobacter* and *Azospirillium*). The best results in this respect were obtained by using magnetic water and *Azotobacter* fertilizer application compared to the control during both experimental season. Furthermore, the highest essential oil content and the percentages of N, P and K were enhanced as well by the combined application of magnetized water and *Azotobacter* application.

Key words: *Majorana hortensis* L. plants, biofertilizers, yield.

INTRODUCTION

In recent decades, the cultivation of medicinal and aromatic plants had noticeably increased and has considered a good source of foreign currency. Improving the productivity of medicinal plants has been the focus of interest for researchers worldwide to avoid the side effects of chemical therapy on human health through utilization of the medical herbs. Furthermore, following the appropriate agricultural practices in growing such crops provide the producers with higher income, in comparison with many other traditional crops (Hassan, 2009).

Marjoram (*Majorana hortensis*, M.) is an aromatic and medicinal perennial plant belongs to Lamiaceae (Labiatae) family that native to Mediterranean region and commonly known as sweet marjoram (Kumar, 2011). Marjoram plant was known to the ancient Egyptians as flavor food and a miraculous herb to cure various diseases. Now the plant is cultivated as one of the important economic export crops for its preservative and medicinal properties. The essential oil isolated from herb used in traditional medicine, as spices in the industry of food, also used in perfumes, cosmetics and pharmaceutical industry for its antibacterial, antimicrobial, antifungal, anti-inflammatory, antidote, anticonvulsant, anti-anxiety anti-gout, antidiabetic, antitumor, Hepatoprotective and antioxidant activity (Burdock, 1995; Tiziana and Dorman, 1998 and Tripathy *et al*., 2017). Bio-fertilizers have beneficial return to increase population of soil microorganisms, especially in the surface layer of root rhizosphere, that create substances which stimulate plant growth. Therefore, it is considered as very important method of providing the plants with their nutritional requirements without having undesirable impact on the environment. It also provides the means for stabilizing soil fertility and has fabulous tendency for decreasing the requirement of chemical fertilizers without compromising on crop yield (Kahil *et al.*, 2017).

The use of the magnetic field as a treatment of water was observed for many industrial purposes. Agricultural and medical terms were used by Shimazaki Seed Company of the magnetic field in improvement of seed

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germination and acceleration of growth. Many studies on using magnetized irrigation water were carried out (Nasher, 2008 and Hozayn and Abdul Qados, 2010), on paulownia ,vegetable crop (Çelik *et al.,* 2008) and chickpea (Maheshwari and Grewal, 2009). Despite the effective role of biofertilizers and magnetic water in improving the productivity of several crops, little is known about the interactive effects of both of them on growth and secondary metabolites accumulation in medicinal plants including sweet marjoram. To the best of our knowledge, few studies have been performed so far to investigate the effect of bio-fertilizers interacted with magnetic water on medicinal plants. So, the objective of this experiment was to investigate the effect of bio-fertilizers and magnetic water treatments on the growth, yield and essential oil content of sweet marjoram.

MATERIALS AND METHODS

This investigation was carried out at the experimental farm of the faculty of Agriculture, Menoufia University at Shiben El-Kom during tow growing the seasons of 2017 and 2018. The aim of this study was investigate the effect of bio-fertilization with N_2-fixing bacterial strains as well as irrigation with magnetized water on the growth, yield and essential oil content of *Majorana hortensis* L. plants

The seedlings were obtained from Medicinal and aromatic plants Department, Horticulture Research Institute, Ministry of Agriculture, Egypt and transplanted at the Experimental Farm on 12th of April in both seasons. During soil preparation, constant doses of calcium super phosphate 150 kg/fed (15.5% P_2O_5) and 100 kg/fed potassium sulphate (48% K_2O) were added in each growing season.

The bacterial strains were obtained from National Research Center, Giza, Egypt. The bio-fertilizers were added in two equal side dressings to the soil in each experimental season and the treatments were as follows:

1. Control without bio (B0)
2. Biofertilization with Rizobium (B1).
3. Biofertilization with Azospirillium (B2).
4. Biofertilization with Azotobacter (B3).

The magnetized water was obtained by passing the water through magnetron tube of 2 inches diameter and 4000 Gauss's strains according to Amin and Qaseem (2009). The previous biofertilizer treatments were applied under either normal irrigation water (M0) or magnetized water (M1). The treatments of this experiment were arranged in spilt-spilt design with three replicates for each treatment. The plants were cultivated at 50 cm apart and the plant density was 16000 plant/fed.

In each experimental season the plants were harvested three times. The plants were cut off about 15 cm above the soil surface. The first cut was done at 12th of June, while the second and the third cuts were performed by the end of August and October, respectively in each growing season. For each cut the following data were recorded:

Growth and yield parameters:

- Plant height in cm.
- Number of branches /plant.
- Fresh weight of herb/plant (g).
- Dry weight of herb/plant (g).

Essential oil determination:

The essential oil percentage was determined in fresh herb samples collected from each cut in both seasons according to the method described in British Pharmacopea (1963). The essential oil percentage (v/w) was calculated according to the following equation:

$$\text{Essential oil percentage} = \frac{\text{Observed oil in graduated tube (mL)}}{\text{Weight of sample (g)}} \times 100$$

The essential oil yield / plant (mL) and per fed (L) were then calculated.

Chemical analysis of herb:

The leaves were oven dried at 70ºC for 72 hours until constant weight and kept as powder for chemical analysis.

- Mineral content:

Nitrogen was determined by Kjeldahl procedure (Bremmer, 1996). Phosphorus was determined by using Spectophotometer (Richards, 1954). Potassium was determined by using Flame photometer (Wettstein, 1957).

Statistical analysis:

The obtained results were subjected to statistical analysis and analysis of variance (ANOVA) was performed using Michigan Statistical Program Version C (MSTATC). Means were compared by Least significant difference (L.S.D.) value at 0.05 probability level as mentioned by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1. Vegetative growth:

1.1. plant height and number of branches:

The recorded data in Table (1) indicate that, the *Majorana hortensis* L. plants grown under different kinds of bio-fertilizers and irrigated with magnetized water were taller than those grown under the individual application of them in the two experimental seasons. The tallest plants were obtained by the combined treatment of Azotobacter and magnetized water (M1 × B2) that reached in the third cut to 45.2 and 40.6 cm in the first and second seasons, respectively. Contrary, the control plants (M0 × B0) resulted in the shortest plants and recorded in the third cut 27.3 and 27.8 cm in both seasons, respectively. In the same line, the interaction treatment of magnetized water and biofertilizers increased the branch number in the two experimental seasons. The highest number of branches / plant was obtained in the third cut of the two growing seasons. The highest branch number was obtained by the combined treatment of Azotobacter and irrigation with magnetically treated water (M1 × B2). By applying this treatment, the branch number was 29.7, 44.1 and 44.9 in the first season while, it was 31.3,41.8 and 47.3 in the second one for the three cuts, respectively.

Increasing the growth due to biofertilizers may be ascribed to the role of such bacterial strains in increasing the endogenous phytohormones such as GA_3, IAA and cytokinins and consequently stimulated cell division and building more vascular tissues. Such effect has been previously reported in *Nigella sativa* (Harb *et al.*, 2011) *Carum carvi* (Mazrou, 2013), *Foeniculum vulgare* (Kusuma *et al.*, 2019) and *Origanum vulgare* (Nikou *et al.*, 2018).

Table (1). Effect of interaction between irrigation with magnetized water and bio-fertilization on plant height and number of branches of *Majorana hortensis* L. plant during 2017 and 2018 growing seasons.

Treatments		Plant height (cm)						Number of branches/plant					
		The first season (201٧)			The second season (2018)			The first season (2017)			The second season (2018)		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
M0	B0	23.2	25.5	27.3	22.5	25.8	27.8	13.2	24.2	25.1	17.0	24.0	26.3
	B1	28.2	28.5	29.8	26.8	30.4	30.8	17.5	27.7	30.8	19.5	28.8	32.9
	B2	35.2	34.4	35.8	32.7	34.6	35.4	23.9	37.0	37.8	22.9	35.9	40.6
	B3	31.5	32.2	32.7	29.4	31.6	33.3	22.3	31.2	32.8	20.0	32.0	35.8
M1	B0	28.9	31.9	32.5	29.3	29.6	30.8	19.1	29.7	30.6	19.8	29.2	31.9
	B1	31.3	35.0	35.8	33.2	33.5	34.2	23.3	34.1	35.3	22.8	34.4	38.1
	B2	40.6	42.3	45.2	37.8	39.6	40.6	29.7	44.1	44.9	31.3	41.8	47.3
	B3	34.9	39.8	39.6	34.4	36.8	37.2	27.7	39.4	41.5	28.2	37.7	43.9
L.S.D (5%)		1.44	1.71	1.33	1.37	0.83	0.78	1.46	1.66	0.62	1.42	1.4	0.76

M0 = not magnetized water, M1 = magnetized water, B0 = without biofertilizers; B1 = Rhizopium; B2 = Azotobacter; B3 = Azospirillum.

Additionally, these results may be explained by the role of magnetized water on increasing the soil content from available N, P and K and also enhanced their absorption by plant roots, which was reflected in promoting plant growth parameters (Midan and Tantawy, 2013). Similar trend has been previously observed in *Origanum majorana* (Khater, 2019), *Rosmarinus officinalis* (Zedan *et al.*, 2020) and *Majorana hortensis* (Khater, 2020). The increment in plant height and number of branches as a result of applying the combined treatment of magnetized water and biofertilzers could be explained through the effective role of both of them in increasing the plant height when solely applied. These results are in agreement with Khater (2019), who reported the same promotion effect of magnetized water and biofertilzers on plant height and number of branches.

1.2. Fresh and dry weight:

Data presented in Table (2) show that the herb fresh and dry weight per plant were positively influenced by the combined application of magnetized water and biofertilizer treatments in the two experimental seasons. All biofertilizers used considerably increased the herb fresh and dry weights compared to the control whether plants irrigated with magnetized water or ordinary one; however, the increment by magnetized water was significantly higher. This trend was observed in both seasons in the three cuts. The most effective interaction treatment was M1 × B2 which maximized the herb fresh and dry weights per plant. By applying this treatment, the total fresh herb yield was 338.1 and 308.4 g/plant while the untreated plants recorded 154.7 and 159.2 g/plant in the two seasons, respectively. The same direction was observed concerning the dry herb yield since the herb yield under M1 × B2 treatment was increased by 132.28 and 130.32% relative to the control in the first and second seasons, respectively. These results are inagreement with those of Khater (2020) who reported that the interaction between magnetized water and biofertilizer increased the fresh and dry weight of herb in *Majorana hortensis* L. plants. Furthermore, the same trend was observed in servel aromatic herbs like *Origanum majorana* (Khater, 2019), *Rosmarinus officinalis* (Zedan *et al.*, 2020) and *Majorana hortensis* (Khater, 2020).

Table (2). Effect of interaction between irrigation with magnetized water and bio-fertilization on fresh and dry weight of *Majorana hortensis* L. plant during 2017 and 2018 growing seasons.

Treatments		Fresh weight /plant (g)						Dry weight /plant (g)					
		The first season (2017)			The second season (2018)			The first season (2017)			The second season (2018)		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
M0	B0	29.1	51.2	74.4	31.0	55.4	72.8	7.3	20.3	23.5	7.9	15.03	20.5
	B1	33.7	84.5	89.8	36.2	81.8	90.4	9.6	29.3	32.9	9.8	23.05	25.6
	B2	46.2	112.5	124.7	43.1	100.4	110.5	14.2	41.2	41.2	12.5	28.8	35.8
	B3	41.1	99.8	103.2	40.4	92.3	104.03	11.7	34.8	35.6	11.4	26.2	31.3
M1	B0	35.2	88.6	91.8	38.1	81.6	88.8	11.4	29.5	34.7	11.1	25.0	29.6
	B1	41.3	103.5	109.8	43.5	98.5	107.3	15.2	37.9	40.6	12.9	28.4	36.6
	B2	53.7	135.6	148.8	52.2	121.4	134.8	18.5	47.5	52.7	15.03	37.6	47.4
	B3	48.7	118.7	131.6	47.1	110.7	127.5	15.8	43.6	43.9	13.8	31.9	44.7
L.S.D (5%)		4.2	4.28	1.63	0.97	2.15	1.74	0.96	1.89	0.93	0.42	1.37	0.53

M0 = not magnetized water, M1 = magnetized water, B0 = without biofertilizers; B1 = Rhizopium; B2 = Azotobacter; B3 = Azospirillium.

1.3. Essential oil percentage:

From data in Table (3) it could be noticed that the combined treatment of magnetized water and bio-fertilizers improved the essential oil percentage compared to the control in both seasons. All biofertilizer treatments produced higher essential oil percentage when applied under magnetized water than under the ordinary water in both seasons Among all interaction treatments, M1 × B2 treatment resulted in the highest essential oil percentage in the two growing seasons. Plants that received this treatment recorded 58.66 and 78.04% higher essential oil percentage relative to the control (M0 × B0) in the second cut during the first and second seasons, respectively. The improvement in the essential oil percentage as a result of the combined application of magnetized water and biofertilizer treatment might be due to the role of magnetized water in inducing enhancement in the mechanism of metabolic process in the plant which was reflected in improving the biosynthesis of essential oil in the leaves (Esfandiar *et al.*, 2012). Furthermore, the important role of the bacterial strains in stimulating the endogenous phytohormone that participating in essential oil metabolism may explain this increase in essential oil. This increase may be ascribed to the role of magnetized water in inducing enhancement in the mechanism of metabolic process in the plant which was reflected in improving the biosynthesis of essential oil in the leaves (Esfandiar *et al.*, 2012). Additionally, the bacterial strains in biofertilizer stimulates the endogenous phytohormone that participating in essential oil metabolism (Youssef *et al.*, 2004). Similar trend has been observed by Abido *et al.* (2011) on spearmint, Nikou *et al.* (2018) on *Origanum vulgare* and Zedan *et al.* (2020) on *Rosmarinus officinalis*.

1.4. Essential oil yield per plant:

From data in Table (4) it could be noticed that the combined treatment of magnetized water and bio-fertilizers improved the essential oil yield per plant compared to the control in both seasons. The biofertilizer treatments produced higher essential oil yield when applied under magnetized water than under ordinary one. Among all interaction treatments, M1 × B2 treatment resulted in the highest essential oil yield (mL/plant) in the two growing seasons. Plants those received this treatment recorded 364.6 and 294.9% higher essential oil yield / plant compared to the control (M0 × B0) in the second cut during the first and second seasons, respectively.

Table (3). Effect of interaction between magnetized water and bio-fertilization on essential oil percentage of *Majorana hortensis* L. plant during 2017 and 2018 growing seasons.

Treatments		The first season (2017)			The second season (2018)		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut
M0	B0	0.57	0.75	0.33	0.29	0.41	0.28
	B1	0.71	0.89	0.49	0.40	0.50	0.40
	B2	0.87	1.07	0.68	0.67	0.71	0.53
	B3	0.75	0.95	0.58	0.63	0.65	0.48
M1	B0	0.67	0.94	0.50	0.46	0.48	0.39
	B1	0.84	1.08	0.66	0.55	0.57	0.59
	B2	1.05	1.19	0.82	0.76	0.73	0.71
	B3	0.89	1.11	0.73	0.69	0.68	0.66

M0 = not magnetized water, M1 = magnetized water, B0 = without biofertilizers; B1 = Rhizopium; B2 = Azotobacter; B3 = Azospirillium.

Table (4). Effect of interaction between irrigation with magnetic water and bio-fertilization on essential oil yield in ml / plant of *Majorana hortensis* L. plant during 2017 and 2018 growing seasons.

Treatments		The first season (2017)			The second season (2018)		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut
M0	B0	16.54	38.27	24.55	9.22	22.44	20.57
	B1	23.97	75.84	44.23	14.57	41.11	36.39
	B2	40.15	119.81	84.79	28.77	71.03	54.87
	B3	30.93	94.56	59.34	25.25	60.23	49.67
M1	B0	23.48	86.29	44.52	17.53	38.96	35.08
	B1	34.55	118.04	68.05	23.71	55.65	64.11
	B2	56.06	177.82	110.51	39.41	88.62	95.37
	B3	43.09	145.75	86.35	32.49	74.72	84.47
L.S.D (5%)		2.54	2.19	1.001	1.28	1.42	0.76

M0 = not magnetized water, M1 = magnetized water, B0 = without biofertilizers; B1 = Rhizopium; B2 = Azotobacter; B3 = Azospirillium

Increasing the essential oil yield due to the combined application of magnetized water and biofertilizer treatment might b2e explained through the dual role of both treatments in inducing the mechanism of metabolic process in the plant which reflected in improving the biosynthesis of essential oil in the leaves (Esfandiar *et al*., 2012). Furthermore, inoculation with bacterial strains as biofertilizer stimulates the endogenous phytohormone that participating in metabolism of essential oil and therefore the essential oil yield was increased. These results are in agreement with those of Abido *et al*. (2011) on spearmint, Zedan *et al.* (2018) on lavender, Nikou *et al.* (2019) on *Origanum vulgare* and Zedan *et al.* (2020) on *Rosmarinus officinalis*.

2. Chemical constituents:

2.1. Total N, P and K percentage:

From data in Tables (5 and 6) it could be noticed that the combined treatment of magnetized water and bio-fertilizers improved the total N, P and K percentage compared to the control in both seasons, the biofertilizer treatments produced higher total N, P and K percentage when applied under magnetized water than under ordinary water. Among all interaction treatments M1 × B2 resulted in the highest total mineral content percentage in the two growing seasons. Plants that received this treatment recorded 48.08 and 47.24% higher total nitrogen percentage to the control (M0 × B0) in the second cut during the first and second seasons, respectively. By applying this treatment the phosphorus percentage was 0.58, 0.60 and 0.48% in the first season while, it was 0.34,0.41 and 0.29% in the second one for the three cuts, respectively. Additionally, plants that received this treatment recorded 21.58 and 49.21% higher potassium percentage to the control (M0 × B0) in the second cut during the first and second seasons, respectively.

These results are in agreement with those obtained by Al-Nimer *et al.* (2011) who found that the magnetized treated water was an effective solvent and has ability to dissolve the nutrient elements easier and faster than purified water (non-magnetized treated). Additionally, biofertilizer led to an increase in mineral content in several aromatic species such as *Hibiscus sabdariffa* (Mazrou, 2013), *Nigella sativa* (Khalid and Mahmoud, 2015) and *Majorana hortensis* (Kandeel *et al.,* 2016). Similar results have been reported also by Ghatas and Mohamed (2018) on *Cymbopogon citrates,* Khater (2019) on *Origanum majorana,* Nikou *et al.* (2018) on *Origanum vulgare* L. and Khater (2020) on *Majorana hortensis* L.

Table (5). Effect of interaction between irrigation with magnetized water and bio-fertilization on total N, P and K percentages of *Majorana hortensis* L. plant during the growing season of 2017.

Treatments		The first season (2017)								
		First cut			Second cut			Third cut		
		N%	P%	K%	N%	P%	K%	N%	P%	K%
M0	B0	1.32	0.29	1.18	1.56	0.38	1.39	1.36	0.23	1.05
	B1	1.57	0.35	1.34	1.79	0.42	1.45	1.53	0.28	1.15
	B2	1.86	0.41	1.47	2.09	0.51	1.55	1.82	0.34	1.36
	B3	1.64	0.36	1.34	1.87	0.44	1.45	1.66	0.29	1.29
M1	B0	1.67	0.37	1.45	1.9	0.47	1.46	1.59	0.34	1.16
	B1	1.84	0.52	1.55	2.08	0.54	1.56	1.88	0.42	1.33
	B2	2.11	0.58	1.68	2.31	0.60	1.69	2.12	0.48	1.53
	B3	1.94	0.55	1.55	2.08	0.56	1.59	1.91	0.45	1.38

Table (6). Effect of interaction between irrigation with magnetized water and bio-fertilization on total N, P and K percentages of *Majorana hortensis* L. plant during the growing season of 2018.

Treatments		The second season (2018)								
		First cut			Second cut			Third cut		
		N%	P%	K%	N%	P%	K%	N%	P%	K%
M0	B0	1.23	0.22	1.25	1.27	0.29	1.26	1.15	0.20	1.29
	B1	1.45	0.26	1.38	1.50	0.33	1.47	1.41	0.23	1.47
	B2	1.64	0.29	1.58	1.69	0.36	1.73	1.62	0.27	1.64
	B3	1.50	0.28	1.51	1.58	0.34	1.65	1.52	0.24	1.54
M1	B0	1.41	0.26	1.48	1.41	0.34	1.54	1.31	0.23	1.39
	B1	1.65	0.30	1.66	1.65	0.38	1.69	1.52	0.26	1.59
	B2	1.86	0.34	1.82	1.87	0.41	1.88	1.72	0.29	1.87
	B3	1.69	0.31	1.73	1.74	0.38	1.75	1.62	0.27	1.78

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التأثير المتكامل للماء المُمغنط والأسمدة الحيوية لتحسين إنتاجية نبات البردقوش

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الملخص العربى

أُجريت هذه الدراسة بمزرعة كلية الزراعة بشبين الكوم جامعة المنوفية خلال موسمي ٢٠١٧/٢٠١٦، ٢٠١٨/٢٠١٧ وذلك بهدف دراسة تأثير بعض المعاملات الزراعية مثل الري بالماء المُمغنط والتسميد الحيوي إما بصورة مُنفردة أو مُتداخلة على صفات النمو الخضري وإنتاج الزيت الطيَّار، وكذلك بعض المُكونات الكيمائية في نباتات البردقوش. وقد تم في هذه الدراسة ري النباتات إما بالماء العادي أو الماء المُمغنط (عن طريق مرور الماء فى أنبوب قُطره ٢ بوصة وشدته ٤٠٠٠ جاوس)، بالإضافة إلى استخدام ثلاث أنواع من الأسمدة الحيوية (الريزوبيم، الأزوتوباكتر، الأزوسبيريلليم) المُحتوية على أنواع بكتيرية مُثبتة للأزوت. وقد صُمِّمت التجربة في قطع مُنشقة. وقد تم تحليل النتائج المُتحصل عليها إحصائياً، وقد أظهرت نتائج تلك الدراسة إلى تحسُّن واضح في كلٍ من صفات النمو الخضري (ارتفاع النبات - عدد الأفرع – الوزن الطازج والجاف للنبات) نتيجة لري النباتات بالماء المُمغنط مع استخدام السماد الحيوي (الأزوتوباكتر).

كما أوضحت النتائج أيضاً تحسُّن كلٍ من مُحتوى نبات البردقوش من الزيت الطيار وإنتاجيته للنبات، كما تحسَّن أيضاً بصورة واضحة مُحتوى النبات من العناصر الغذائية (النيتروجين، الفوسفور، البوتاسيوم) نتيجة لري النباتات بالماء المُمغنط مع استخدام السماد الحيوي (الأزوتوباكتر) وذلك خلال موسمي النمو، بينما سجلت مُعاملة الكنترول المُتمثلة في الري بالماء العادي وبدون استخدام أسمدة حيوية خلال موسمي النمو أقل القياسات الخضرية والمحصول، وكذلك المُكونات الكيماوية للنبات.