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IMPACT OF FOLIAR APPLICATION WITH SOME BIO AND CHEMICAL STIMULANTS ON GROWTH, YIELD AND SOME ENDOGENOUS HORMONES OF CUCUMBER PLANTS (*Cucumis sativus* L.)

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ABSTRACT: ***There is a recent tendency to use biostimulants in horticultural crops as natural and safe substances. Two field experiments were carried out in a private farm, Kafr El Shiekh Governorate, Egypt during the two successive winter seasons of 2006/07 and 2007/08. The objective of the present study was to evaluate the impact of foliar application of some stimulatnts such as humic acid, amino acids, ascorbic acid, salicylic acid and dry yeast as well as hydrogen peroxide (H_2O_2), which generally regarded as safe compounds, on the growth, yield and fruit quality as well as hormones and N, P, and K contents in cucumber leaves. Spraying with H_2O_2, dry yeast and amino acids significantly increased vegetative growth characters. Chlorophyll content was improved by stimulants applications. All foliar application treatments produced higher early and total yields than control, the best results were obtained from humic acid, dry yeast, and H_2O_2. Moreover, ascorbic acid content was increased with spraying both of ascorbic acid and H_2O_2. Endogenous hormones contents were increased by H_2O_2 (GA_3, IAA and Kinetin), humic acids (IAA and GA_3) and dry yeast (Kinetin). Also, applying most of stimulants statistically increased the contents of N % (yeast and amino acids), P % (yeast and H_2O_2) and K % (H_2O_2 and amino acids).Therefore, the use of H_2O_2, dry yeast and humic acid as foliar application on cucumber plants have a good potential strategy to improve the growth, yield and fruit quality.***

Key words: ***Cucumber, biostimulants, H_2O_2, hormones***

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable crop for the greenhouse industry around the world. The cucumber fruit products are used not only for fresh eating and culinary cooking, but also for salad and pickling. The total cultivated area of cucumber in Egypt was about 92 000 hectares in 2007 according to the statistics of FAO.

There is a recent tendency to use biostimulants which promote plant growth and being used increasingly in horticultural crops. Biostimulants are

defined by Russo and Berlyn (1990) as being " non-fertilizers which benefit plant growth" containing natural products such as humic, amino, ascorbic and salicylic acids, yeast, kinetin, seaweed extracts, trace elements …etc. They can reduce plant stress and enhance plant nutrient uptake as well as decreasing the need for inorganic fertilizer for plant growth and development.

Nowadays, biostimulants have become commonly used as foliar spray for horticultural crops as natural and safe substances. The favorable effect of biostimulants was reported on many crops. Humic acid is one of the major components of humic substances. Moreover, humic acids have also been shown to stimulate plant growth and improve crops yield and quality (Atiyeh *et al.,* 2002 on tomato).

Amino acids are precursors or activators of phytohormones and growth substances, which as organic nitrogenous compounds are the building block in the synthesis of proteins (Davies, 1982). Therefore, amino acids have a regulatory role in stimulating and promoting productivity of many vegetable crops such as tomato (Cohen *et al*., 1982), pepper (Talaat, 2003), cucumber (Duan *et al*., 2008) and eggplant (El-Tohamy et al., 2008).

Foliar sprays of ascorbic acid have a stimulatory effect on plant growth and yields of some vegetables (El-Greadly, 2002 on cucumber and El-Banna *et al*., 2006 on potato).

Salicylic acid (SA) is a natural signal molecule, which plays an important role in regulating a number of physiological processes and plant resistance to biotic and abiotic stresses (He *et al*., 2005). Obvious positive effects of SA application have been achieved on growth and yield of cucumber (Shi and Zhu, 2008).

Additionally, yeast becomes commonly used as biostimulants and it is considered as a natural source of kinetin and it has stimulative effects on plants. In this manner, spraying plants by dry yeast produced vigorous vegetative growth and large yields for some vegetables (Abou El-Nasr *et al*., 2001 on squash, Hussain and Khalaf, 2007 on potato and El-Tohamy *et al*., 2008 on eggplant).

Hydrogen peroxide is an environmentally friendly compound and considered as a GRAS (Generally Regarded As Safe) as well as allowed in organic crop production (NOP, 2003). There are many uses for H_2O_2 in agriculture; Al-Mughrabi (2007) showed significantly higher emergence % and total yield of potato when treated with H_2O_2. On cucumber, using H_2O_2 increased roots fresh weight (Li *et al*., 2007). Spraying H_2O_2 with pharmaplant turbo decreased the disease severity while increased the early and total yields (Hafez *et al*., 2008).

Hence, the objective of this work was to investigate the impact of foliar application of different biostimulants (humic, amino, ascorbic and salicylic acids, yeast and H_2O_2) on growth, yield and some hormones content in cucumber leaves.

MATERIALS AND METHODS

This investigation has been performed in two successive seasons 2006/07 and 2007/08 in a private farm (Al-Barakah, Al-Riad District, Kafr El-Sheikh Governorate, Egypt) using cucumber hybrid (dp cu 1005 F_1, Holland) grown under plastic houses.

Seedlings were transplanted on the middle of October in both seasons on one side of the ridge at 50 cm spacing between plants within the row. Plant density was 2.5 plants per square meter. Drip irrigation and all cultural practices were applied according to the recommendations of Ministry of Agriculture.

The experiment in each season included seven treatments as follows:

1- **Humic acids (Mega Power, 3 ml/l): Mega Power, as a liquid form contains humic acid (19 %) and fulvic acid (2 %), it produced by AGAS company, Egypt.**
2- **Amino acids (Amino total, 2 ml/l): It contains total amino acids (40%) and free amino acids (17%), it received from Technogreen company, Egypt.**
3- **Ascorbic acid (at 0.3 g/l).**
4- **Salicylic acid (at 0.5 g/l).**
5- **Dry yeast (7.5 g/l): Yeast is a bio-compound locally produced in Egypt. It contained active fungus, 47% protein, 33% carbohydrates, 8% nucleic acids, 4% lipids, 8% minerals and vitamins (Nagodawithana, 1991). It was activated by dissolving the definite amount in warm water (38 ^{0}C), adding sucrose at the same rate, and kept over night before spraying.**
6- **Hydrogen peroxide (H_2O_2 30%) at 20 mM.**
7- **Control (plants with instill water spraying).**

Four foliar applications were performed fortnightly for each stimulant starting one month after transplanting. Spraying was carried out on plants till run off.

These concentrations which mentioned above were applied as a result of preliminary experiment using three levels from each substrate.

The following measurements were recorded:

1- **Plant growth parameters: Plant length (cm), numbers of leaves and leaf area /plant (cm^2) were determined at 60 days after transplanting.**

2- **Chlorophyll in leaves: Relative green color was measured with SPAD meter (Minolta Corp, Ramsey, N.J.) after 60 days from transplanting.**

3- **Fruit yield: Early yield was considered as the weight of fruits per square meter of the first two weeks of harvesting period. Total yield was the weight of all fruits /m^2 at all harvesting time.**

4- **Fruit quality: TSS % was estimated by a hand refractometer, and ascorbic acid content (mg/100 g fw) according to A.O.A.C. (1965).**

5- **Endogenous phytohormones: They were determined in the samples of leaves after seven days from the last spray for each treatment at the laboratory of Arid Land Agricultural research and services Center Faculty of Agriculture- Ain Shams University Egypt. Auxin (IAA) (mg/100g), Gibberellic acid (GA_3) (mg/100g) and kinetin (µg/100g) were determined (in the second season) as the method described by Badr *et al.* (1971).**

6- **Nitrogen, phosphorus and potassium contents in cucumber leaves: They were estimated according to FAO (1980).**

Statistical analysis:

The experimental design was established as a complete randomized blocks design with 4 replicates. Data were tested by analysis of variance (Little and Hills, 1972). Duncan`s multiple range test was used for comparison among the treatment means (Duncan, 1965).

RESULTS AND DISCUSSION

1- Growth parameters:

The growth parameters as affected by chemical and biostimulants treatments are shown in Table (1). All growth parameters, i.e., plant length, number of leaves/plant and leaf area/plant, were significantly improved by most of treatments as compared with the control. Using dry yeast, H_2O_2 and amino acids as foliar application generally produced the best results of all growth parameters in both seasons. The lowest growth values were resulted from control plants and salicylic acid spraying which didn't statistically differ with the control.

The favorable effect of yeast on plant growth might be attributed to the fact that yeast application increased the uptake and contents of N, P and K in plants (Abou El-Nasr *et al.*, 2001) as shown also in Table (3). Dry yeast

considers as a natural source of kinetin (Tourky *et al*., 2001) and that enhance cell division and enlargement, and consequently increasing leaf surface area.

In addition, the vigorous growth of cucumber plants caused by hydrogen peroxide spraying may be attributed to Reactive Oxygen Species (ROS) that affect the morphogenetic responses of cells and plant explants (Benson and Roubelakis, 1994). It increased also kinetin content in cucumber leaves (Table, 3) which enhance plant growth. H_2O_2 as a signaling molecule, it is a key regulator of plant responses to range of endogenous signal and stimuli such as auxins (Li *et al*., 2007) which plays a central role in numerous development elongation and differentiation (Rout, 2006). In this manner, Simonovicova *et al*., (2004) stated that H_2O_2 can be involved in promoting the root growth of cucumber. These results are in agreement with the results of Li *et al*. (2007) and El-Tohamy *et al*. (2008).

Also, the good results of amino acids may be due to regulatory role in stimulating and promoting productivity of plants (Talaat,2003 and Duan *et al*., 2008)

Table (1): Effect of bio and chemical stimulants applications on some vegetative growth parameters of cucumber plants in both seasons.

Treatments	Plant length (cm)	No. of leaves /plant	Leaf area (cm^2)/plant	Plant length (cm)	No. of leaves /plant	Leaf area (cm^2)/plant
	2006/07 season			2007/08 season		
Humic acids	61.1 bc	28.4 bc	121.8 b	61.9 bc	25.9 ab	138.8 ab
Amino acids	64.9 ab	26.8 c	128.6 ab	64.6 b	27.9 a	135.0 abc
Ascorbic acid	60.2 bc	24.6 d	116.9 b	63.1 bc	24.1 bc	128.3 bc
Salicylic acid	56.8 cd	26.8 bc	113.7 b	60.1 cd	23.7 bc	131.4 bc
Dry yeast	68.3 a	29.5 ab	129.7 ab	63.2 bc	27.5 a	138.6 ab
H_2O_2	65.9 a	30.9 a	144.3 a	70.6 a	27.9 a	145.8 a
Control	54.0 d	23.4 d	114.8 b	57.7 d	23.3 c	125.6 c
F. test	**	**	*	**	**	*

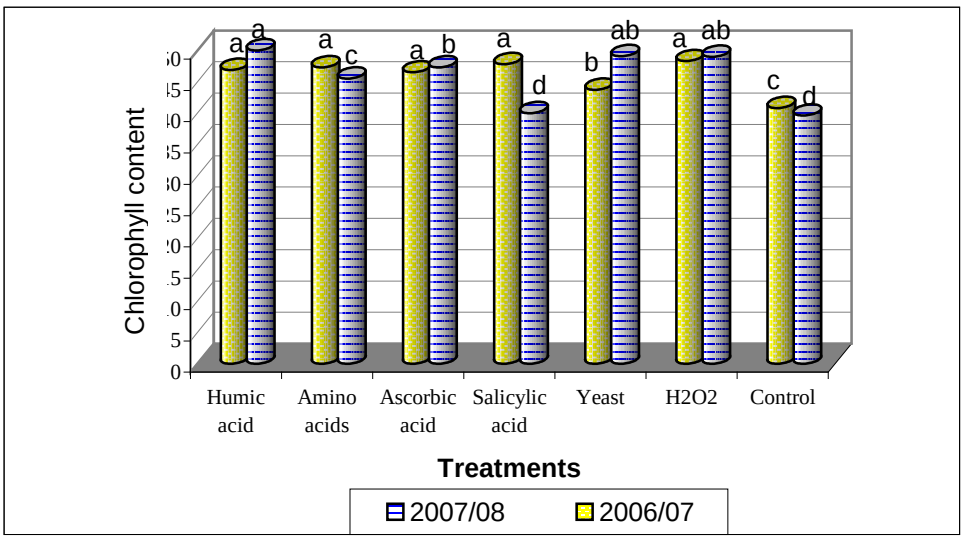
Means designed by the same letter are not significantly difference at the 5% level according to Duncan`s test.

2- Chlorophyll content:

Data presented in Fig. (1) show that, foliar application of all treatments caused a significant increase in the chlorophyll content compared to that of control plants in both seasons. Plants sprayed with humic acids, H_2O_2 or yeast had the highest chlorophyll content, while untreated plants gave the lowest values in most cases. Using both of amino and ascorbic acids increased chlorophyll content without significant difference of each other in the first season.

The superiority of most treatments in chlorophyll content may be due to their stimulative effects. Therefore, H_2O_2 increased chlorophyll values (Benson and Roubelakis, 1994), yeast also stimulate the synthesis of chlorophyll (Castelfranco and Beale, 1983). The positive influence of humic acids could be mainly due to hormone-like activators of humic acids through their involvement in photosynthesis (Muscolo *et al*., 1999).

The present results are in agreement with those of Abou El-Nasr *et al.* (2001) on squash, Atiyeh *et al*. (2002) on cucumber and El-Tohamy *et al*. (2008) on eggplant.



Means designed by the same letter are not significantly difference at the 5% level according to Duncan`s test.

Fig. (1): Effect of bio and chemical stimulants applications on chlorophyll content of cucumber leaves in both seasons.

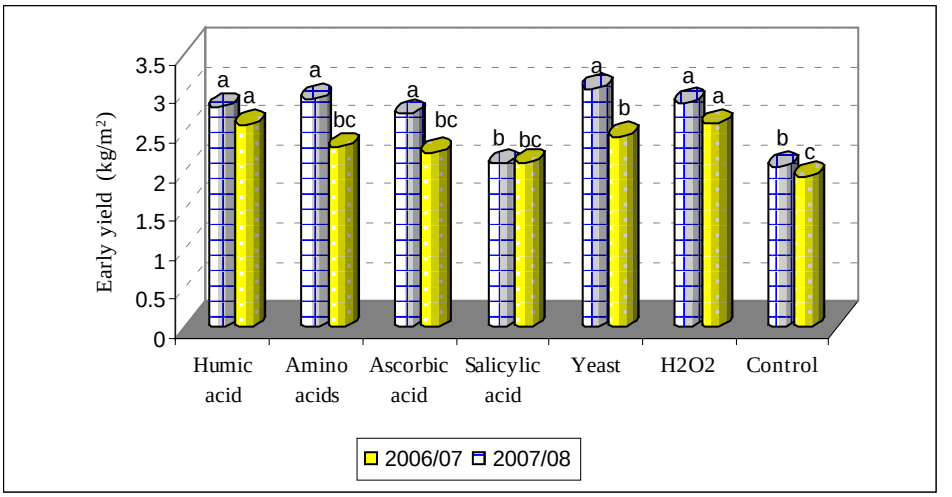
3- Fruit yield:

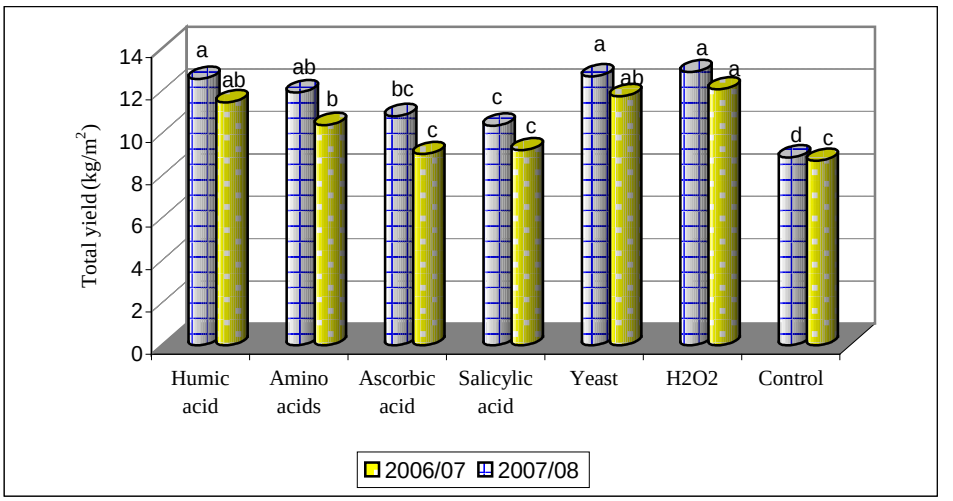
It is clear from Fig. (2) that, spraying cucumber plants by all stimulants increased early and total yields over control. The differences were significant at the two seasons. Meanwhile, applying dry yeast, H_2O_2, humic and amino acids produced the highest early and total yields in most cases. In contrast, salicylic acid spraying showed the lowest values without significant difference of the control plants.

The stimulative effect of dry yeast on yield may be due to its effects on plant growth (Table, 1) and chlorophyll content (Fig. 1) which correlated positively with the productivity. Furthermore, yeast contained some stimulative substances i.e., kinetin (Tourky, 2001) and vitamins (Nagodawithana, 1991) which might be played a considerable role in orientation and translocation of metabolites from leaves into the productive organs and consequently lead to the improvement of cucumber yield.

The greater cucumber yield with spraying humic acids may be attributed to enhancing the growth (Table, 1) which mainly due to hormone-like activities of humic acids through their involvement in cell respiration, photosynthesis, protein synthesis and various enzymatic reactions (Muscolo *et al*., 1999 and Atiyeh *et al*., 2002).

The performed higher yields from H_2O_2 application might be due to its role for improving growth (Table, 1 and Fig. 1). Also, H_2O_2 has a wide-ranging effects in many biological systems, it has also a role in plant defense responses to pathogens and abiotic stresses (Alvarez *et al*., 1998). All of these may occur and lead to the improvement of cucumber yield.





Means designed by the same letter are not significantly difference at the 5% level according to Duncan`s test.

Fig. (2): Early and total yields of cucumber plants as affected by foliar applications of chemical and biostimulants during both seasons.

The higher values of early and total yields achieved by using amino acids are mainly attributed to their regulatory role in promoting the growth and productivity of many plants (Cohen *et al*., 1982 on tomato and Talaat,

2003 on cucumber). Similar results were obtained by Abou El-Nasr *et al.* (2001), Atiyeh *et al*. (2002), Hussain and Khalaf (2007) and Hafez *et al*. (2008).

4- Fruit quality:

The results in Table (2) show that spraying cucumber plants with most of used stimulants led to increas ascorbic acid content compared to control plants, especially in the first season. However ascorbic acid and H_2O_2 application tended to produce the highest values followed by yeast application. The lowest values were obtained from control plants and with humic acid application.

The increment of ascorbic acid content by above stimulants may be due to increasing the enzyme activities in fruits (Bayoumi, 2008).

Table (2): Effect of bio and chemical stimulants applications on ascorbic acid content and TSS % of cucumber fruits in both seasons.

Treatments	Ascorbic acid (mg/100g f.w.)	TSS (%)	Ascorbic acid (mg/100g f.w.)	TSS (%)
	2006/07 season		2007/08 season	
Humic acids	14.83 c	4.22	14.16 d	4.40
Amino acids	14.69 c	4.24	15.73 bcd	4.52
Ascorbic acid	17.32 ab	4.28	18.10 a	4.34
Salicylic acid	16.14 bc	4.70	14.98 cd	4.32
Dry yeast	16.50 b	4.24	16.70 abc	4.44
H_2O_2	18.09 a	4.44	17.57 ab	4.32
Control	13.52 d	4.32	14.61 cd	4.34
F. test	**	NS	**	NS

Means designed by the same letter are not significantly difference at the 5% level according to Duncan`s test.

Concerning TSS %, data in Table (2) clear that there were no significant differences among treatments in both seasons. These results are in agreement with those of Abou El-Nasr *et al.* (2001), Bayoumi (2008) and El-Tohamy *et al*. (2008).

5- Endogenous hormones:

Data in Table (3) indicate that, endogenous hormones (GA_3, IAA and kinetin) were significantly influenced by different stimulants. The higher contents of estimated hormones were observed in plants with application of H_2O_2 followed by humic acids in case of GA_3 and IAA or yeast application in case of kinetin. On the contrary, control plants showed the lowest values in all hormones content, H_2O_2 as a reactive oxygen and regulator may promote cucumber growth and productivity through the increase of endogenous hormones (Table, 3) and enzyme activities (Bayoumi, 2008).

Table (3): Effect of bio and chemical stimulants applications on some endogenous hormones and N, P and K contents of cucumber plants in the second season.

Treatments	GA_3 (mg/100gm fw.)	IAA (mg/100gm fw.)	Kinetin (µg/100g fw.)	Nutrients (%)		
				N	P	K
Humic acids	67.23 b	1.19 a	697.8 f	4.8 c	0.568 b	2.15 cd
Amino acids	29.77 e	0.34 c	1190.1 e	5.8 a	0.590 b	3.17 a
Ascorbic acid	51.50 c	0.35 c	1520.9 d	5.5 b	0.573 b	2.29 c
Salicylic acid	39.87 d	0.77 b	1634.7 c	4.1 d	0.575 b	2.11 d
Dry yeast	8.86 f	0.69 b	3036.4 b	5.8 a	0.643 a	2.64 b
H_2O_2	123.04 a	1.20 a	3624.7 a	5.5 b	0.660 a	3.17 a
Control	8.76 f	0.33 c	601.41 f	4.0 d	0.533 c	2.29 c
F. test	**	**	**	**	**	**

Means designed by the same letter are not significantly difference at the 5% level according to Duncan`s test.

6- Nutrient contents in leaves:

Data presented in Table (3) show that, the nutrients (N, P and K) in the leaves were significantly affected by different stimulants.

Concerning N%, the highest values were obtained from amino acids and yeast applications, followed by ascorbic acid and H_2O_2. The highest values of P% were obtained from plants spraying with H_2O_2 and dry yeast. Dealing with K%, application of H_2O_2 and amino acids caused the highest values, followed by yeast application.

Applying of salicylic acid or spraying with distill water (control) showed the lowest values of the previous characters in most cases. These results are in harmony with the findings of Abou El-Nasr *et al.* (2001), El-Banna *et al*. (2006) and El-Tohamy *et al*. (2008).

Application of H_2O_2, Humic acids and yeast resulted in an increase in both of endogenous promoting hormones and nutrients content in leaves which caused an increase in the growth parameters and consequently increased the early and total yields of cucumber plants.

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تأثير الرش ببعض المنشطات الحيوية والكميائية على النمو والمحصول وبعض الهرمونات الداخلية لمحصول الخيار.

***يسرى ابو المكارم بيومى **منال عبد الرحمن عبد الله **احمد قطب حاتم**

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****قسم الخضر الخلطية التلقيح – معهد بحوث البساتين – مركز البحوث الزراعيه – مصر.**

الملخص العربى

يعتبر استخدام منشطات النمو الطبيعية الآمنة فى إنتاج المحاصيل البستانية من الاتجاهات الحديثة للإنتاج. أجريت دراسة خلال موسمين شتويين ٢٠٠٦/٢٠٠٧ و٢٠٠٧/٢٠٠٨ على محصول الخيار بمزرعة خاصة بمحافظة كفر الشيخ بهدف دراسة تأثير الرش ببعض منشطات النمو الامنه (حمض هيومك – الأحماض الأمينية – حمض اسكوربيك – حمض سلسيليك – الخميره الجافه – فوق اكسيد الهيدروجين) على النمو الخضرى والمحصول و جودة الثمار ومحتوى الأوراق من بعض الهرمونات الداخلية والعناصر. أدى رش النباتات بكل من فوق اكسيد الهيدروجين – الخميره – الحمض الامينى لزيادة صفات النمو الخضرى. كما ارتفع محتوى الاوراق من الكلوروفيل لكل معاملات الرش مقارنة بالكنترول (الرش بالماء المقطر). أدى الرش بالمنشطات المختلفة لتبكير المحصول و زيادة المحصول الكلى مقارنة بالكنترول وأفضل النتائج للمحصول المبكر و الكلى كانت من رش نباتات الخيار بحمض الهيوميك والخميره وفوق أكسيد الهيدروجين مقارنة بالكنترول. أدى الرش بكل من حمض الاسكوربيك و فوق أكسيد الهيدروجين لزيادة محتوى الثمار من حمض الاسكوربيك. كما زادت الهرمونات الداخليه بالرش بفوق اكسيد الهيدروجين (اندول حمض الخليك . جبرلين . كينيتين) ، حمض الهيوميك (اندول حمض الخليك– جبرلين) ،الخميره (كينيتين). كما ادى الرش بالمعاملات المختلفه لزيادة المحتوى من النتروجين (الحمض الامينى . الخميره) ، ومحتوى الفوسفور (فوق اكسيد الهيدروجين – الخميره) ، ومحتوى البوتاسيوم (فوق اكسيد الهيدروجين . الحمض الامينى). يمكن التوصية برش نباتات الخيار بفوق اكسيد الهيدروجين ، او الخميره ، اوحمض الهيومك لتحسين النمو و زيادة المحصول والجوده.

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