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EFFECT OF SOME BIOSTIMULANTS ON GROWTH, YIELD AND VOLATILE OIL CONTENT OF *PIMPINELLA ANISUM* L.

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ABSTRACT: ***This experiment was carried out to investigate the effects of liquorice, chitosan and active dry yeast as biostimulants on the growth, fruit and volatile oil production of anise. Plants were foliar sprayed with liquorice at 20 g/L, chitosan at 400 mg/L and active dry yeast at 6 g/L. Untreated plants were sprayed with tap water. The results revealed that foliar spraying with liquorice, chitosan or dry yeast significantly enhanced the growth characters of anise compared to the control in both the experimental seasons. Additionally, the fruit yield attributes were also considerably improved due to any biostimulant relative to untreated plants. Application of liquorice, chitosan or dry yeast not only enhanced the volatile oil yield but also increased the main components in volatile oil. Besides, photosynthetic pigments, total carbohydrates and N, P, K percentages were considerably increased due to the biostimulants treatments. Generally, the most effective biostimulant was chitosan followed by active dry yeast in two experimental seasons. To stimulate the growth and increase the quantity and quality of fruits and volatile oil yield of anise, plants should be foliar sprayed with either chitosan at 400 mg/L or active dry yeast at 6 g/L.***

Key words: ***Lecorice; chitosan; Yeast, Volatile oil; Chlorophyll; Carbohydrates***

INTRODUCTION

Anise *(Pimpinella anisum* L.), belongs to Apiaceae Family, is an important spice and medicinal plant. Anise is native to Mediterranean region and cultivated worldwide for fruit production which contain 2-3 % volatile oil (Hassan and Ali, 2013). Anise fruits are considered outstanding natural material for cosmetic, pharmaceutics, perfumery and food industries (Ross, 2001). Additionally, anise has gained more attention due to antioxidtaive, antimicrobial and antifungal effects on human (Tepe *et al*., 2006). One of the main factors affecting the productivity of anise is fertilization. Bhuvaneshwari *et al*. (2002) found that chemical fertilizers enhance the growth and fruit productivity of anise. However, excessive use of chemical fertilizers deteriorates soil fertility, creates pollution of agro-ecosystem and increases the production cost.

Recently, the interest of many researchers worldwide is how to produce chemical-free products to guarantee the safety and quality. To optimize the level of applied chemical fertilizers and therefore decrease the pollution some strategies have been developed including usage of untraditional fertilizers and biostimulants (Hassan and Ali, 2013; Ali and Hassan, 2014; Ali *et al*., 2018). Biostimulants are various substances or microorganisms used to improve the growth and development of plants and there is growing scientific evidence which supports its use as agricultural inputs on seveal plant species (Calvo *et al*., 2014). Liquorice root extract, obtained from *Glycyrrhiza glabra*, contains minerals, flavonoids, phenolic compounds, amino acids, vitamins and mevalonic acid which involved in gibberellins synthesis and therefore is considered a plant growth promoter (Moses *et al*., 2002). Foliar application with liquorice water extract not only improved the growth, fruit yield and essential oil content in coriander but also enhanced the oil composition (Abd El-Azim *et al*., 2017). The growth and yield of roselle were considerably improved due to liquorice treatment. Moreover, the total phenolic compounds and scavenging activity were also increased (Hassan and Abd El-Samee, 2015). The impact of liquorice root extract on growth, yield and composition has been reported in several crops (Nasser *et al.,* 2014 on *Trigonella foenum-graecum;* Thanaa *et al.,* 2016 on *Prunus amygdalus*).

Chitosan is a biopolymer, a chitin derivative, a natural antitranspirant, low toxic and inexpensive compound which is environmentally friendly and biodegradable with several uses in agriculture (Malekpoor *et al.,* 2016). Chitosan stimulates vital plant processes from single cells and tissues, through physiological and biochemical processes (Hadwiger, 2013). Chitosan has proved to be operative for plant growth stimulation (Farouk *et al*., 2011; Karimi *et al*., 2012). Zhao *et al*. (2005) reported that chitosan is the most commonly used elicitor for enhancing the accumulation of secondary metabolites in herbs and medicinal plants since it activates genes that motivate the biosynthetic pathways of secondary metabolites (Lei *et al*., 2011). It has been reported that chitosan treatment improved the dry matter and volatile oil productivity in basil (Bistgani *et al*., 2017).

Active dry yeast is considered a plant growth enhancer as a natural cytokinins source that simulates cell division and enlargement as well as the synthesis of protein, nucleic acid and vitamin B (Amer, 2004). It also releases CO_2 which resulted in enhancing net photosynthesis (Kurtzman and Fell, 2005). Furthermore, Khedr and Farid (2000) found that the promotin effect of dry yeast is ascribed to its ability in stimulation of endogenous hormones such as GA_3 and IAA. Yeast extract is among the most commonly elicitors which used for improving the plant growth and productivity as well as secondary metabolites accumulation (Kassem, 2013; Bistgani *et al*., 2017). The positive effects of dry yeast on growth and volatile oil content in several medicinal and aromatic plants were observed (El-Sayed *et al*., 2002 on coriander; Heikal, 2005 on thyme; Sharaf-Eldin *et al*., 2008 on lemon balm; Ezz El-Din and Hendawy, 2010 on *Borago officinalis*). Enhancing the quantity and quality of anise was and still the main goal of several researchers worldwide. However, little information is available concerning the effects of liquorice, chitosan and yeast biostimulants on the growth and productivity of anise. Therefore, the aim of this experiment was to investigate the response of anise plants to those three biostimulants and how can they affect the growth, yield and volatile oil content of anise.

MATERIALS AND METHODS

1. Plant materials and soil analysis

Two field experiments were conducted at Faculty of Agriculture, Menoufia University, Shibin El-Kom (30°33′24.8″N 31°00′51.3″E) during 2017/ 2018 and 2018/2019 seasons. The objective of this study was to investigate the effects of liquorice, chitosan and dry yeast on the growth, fruit yield and volatile oil content of anise plants. Seeds were obtained from Medicinal and Aromatic plants Department, Horticulture Research Institute, Ministry of Agriculture, Egypt. The experimental soil was prepared and divided to 1.8 x 1.8 m plots that contained three rows with 6 hills each. The seeds were sown on October 1st in both seasons. The plants were thinned after three weeks at one plant per hill. The physical properties of the used soil were (14.29 % sand, 40.13 % silt, 45.58 % clay). The chemical properties of soil were (pH, 7.98, OM, 0.17 %, EC, 1.30 dsm^{-1}, SO_4^{-2}, 44.49 ($meqL^{-1}$), Na^+, 2.17 ($meqL^{-1}$), Ca^{+2}, 42.11 ($meqL^{-1}$), HCO_3, 2.03 ($meqL^{-1}$), Cl^-, 0.48 ($meqL^{-1}$), total N^+, PO_4^{-3}, K^+ were 0.18, 0.032 and 0.036 %, respectively). When required, the other cultural practices needed during the growth were done.

2. Biostimulants treatments and experimental design

The dried roots of liquorice were ground in a grinder and passed through a 40 mesh screen. Dried powdered sample (20 g/L) was extracted with distilled water as recommended by Hassan and Abd El-Samee (2015). The solution was then placed in orbital shaker for 24 h at room temperature. Finally, the extract was filtered using Whatman filter paper No.1 and kept for spraying. Chitosan was applied at 400 mg/L as recommended by Malekpoor *et al*. (2016). Chitosan was initially dissolved in acetic acid (5 %) and then diluted in distilled water to the required concentration. Furthermore, active dry yeast was purchased from a local market in a powder form. Dry yeast was applied at 6 g/L as recommended by Sharaf-Eldin *et al.* (2008). Aqueous dry yeast solution was overnight activated before foliar spraying application. The plants were monthly sprayed with the previous biostimulants for three times and the first one was after one month from thinning. Control plants were sprayed with tap water. The experimental design was a randomized complete design (RCD) with three replicates according to Snedecor and Cochran (1980).

3. Growth and fruit yield characters

At harvest stage (when fruits were greenish yellow in color and before fully ripening stage) at the April 10th in both experimental seasons, the plants were harvested. The growth attributes i.e. plant height (cm), branch number (main branches), herb fresh and dry weight/plant (g) were recorded. The fruit yield components i.e. number of umbels/plant, fruit yield/plant (g) and per fed (kg) were assessed.

4. Volatile oil determination

Anise fruits were dried until the weight of the sample remained constant. Dry fruit samples (50 g) were used for determination of the volatile oil percentage. The fruits were milled before distillation. Hydro-distillation was used for volatile oil extraction in a Clevenger apparatus for 3h. The distillation was done in triplicate samples and the given oil contents are the average values. The oil percentage was determined as described in British Pharmacopea (1963), using the following equation:

Volatile oil percentage = oil volume in the graduated tube/weight of sample x 100.

The oil yield (plant & fed) was then calculated. Anhydrous sodium sulfate was used for volatile oil drying then the oil was stored in dark and cool conditions till GC-MS analysis. Samples of essential oil were performed using a Hewlett-Packard 5890 A. The oil components were identified by comparing the mass spectrum and retention times with those of standards, NIST library of the GC-MS system and literature data.

5. Photosynthetic pigments

The photosynthetic pigments in anise leaves were spectrophotometrically assessed according to Metzner *et al.* (1965) method. Fresh leaf samples were randomly collected from the middle part of stem at flowering stage for chlorophyll and carotenoids determination. The values were recorded as mg/g based on fresh weight.

6. Assessment of total carbohydrates

Total carbohydrate percentage was analyzed in anise leaves. Samples were dried in an oven at 70 °C for 24 h according to A.O.A.C. (1980). The obtained fine powder was used to evaluate total carbohydrate percentage as described by Herbert *et al.* (1971).

7. Leaf mineral concentration

Anise herb samples were oven dried at 70 ˚C for 48 hours. Samples were then milled to obtain suitable material for nutrient analysis. Samples (0.5 g) were digested in sulphuric and perchloric acids (Piper, 1967; Jackson, 1978) for mineral content analysis. Nitrogen was determined by the micro-Kjeldhl method according to Black *et al.* (1965), phosphorus were colorimetrically assessed at 660 nm as described by Jackson (1978), while potassium measurement was performed by flame photometer (Jackson, 1978).

8. Statistical analysis

The obtained data were subjected to statistical analysis using Michigan Statistical Program Version C (MSTATC). The analysis of variance (ANOVA) was performed to compare means. Means were separated using Duncan multiple range test at 0.05 level.

RESULTS AND DISCUSSION

1. Vegetative growth parameters

It is evident from data presentedin Table (1) that the vegetative growth characters (plant height, branch number, fresh and dry weights) of anise were significantly enhanced due to applying the different biostimulants compared to the control in both experimental seasons. Spraying with chitosan significantly more improved the growth compared to either liquorice or dry yeast applications and recorded the highest values in this respect in both seasons. The improvement in anise growth due to liquorice extract application could be explained through its content of triterpene saponins, mevalonic acid which concert to gibberellins in plants, vitamins, polysaccharide and several minerals which play important roles in plant growth motivation (Zadeh *et al.*, 2013; Elrys and Merwad, 2017). These results are in harmony with the results of Hassan and Abd El-Samee (2015) on roselle and Abd El-Azim *et al.*, (2017) on fennel.

Plant growth stimulation of chitosan may be attributed to increasing the availability and uptake of water and essential elements through adjusting cell osmotic pressure and increasing antioxidants (Guan *et al.*, 2009). It was found that chitosan increased the activities of key enzymes of nitrogen metabolism and improved the transportation of nitrogen in leaves which improved plant growth and development (Gornik *et al.*, 2008). The current results are in agreement with the results of Malekpoor *et al.* (2016) on basil and Bistgani *et al.* (2017) on thyme. Bitelli *et al.* (2001) reported that chitosan treatment maintained dry matter production partly through a reduction in transpiration by induction of stomatal closure. The improvement in growth characters in the current study due to dry yeast treatment is in accordance with the results of Sharaf-Eldin *et al.* (2008) on lemon balm and Kassem (2013) on rosemary. Additionally, Amer (2004) reported that yeast is a source of cytokinins which simulate cell division and enlargement as well as the synthesis of protein. Such improvement was documented on several medicinal and aromatic plants (El-Sayed *et al.*, 2002 on coriander; Heikal, 2005 on thyme; Ezz El-

Din and Hendawy, 2010 on *Borago Officinalis*).

Table 1. Effects of some biostimulants on vegetative growth characters of anise plant during 2017/2018 and 2018/2019 seasons.

Biostimulants	Plant height (cm)	Branch number/plant	Herb fresh weight (g)	Herb dry weight (g)
		2017/2018 season		
Control (Tap water)	41.37d	4.72d	50.85d	12.37d
Liquorice (20 g/L)	50.24c	6.15c	71.94c	15.33c
Chitosan (400 mg/L)	55.66a	8.27a	78.18a	18.35a
Dry yeast (6 g/L)	52.15b	7.33b	73.76b	16.72b
		2018/2019 season		
Control (Tap water)	42.25d	4.63d	52.15d	12.45d
Liquorice (20 g/L)	51.36c	6.22c	72.84c	15.51c
Chitosan (400 mg/L)	55.87a	8.47a	79.23a	18.49a
Dry yeast (6 g/L)	53.29b	7.39b	74.88b	16.92b

Means had different letters in each season are significantly differ from each other according to Duncan's multiple range test at 5 % ($P \leq 0.05$).

2. Fruit yield

Data presented in Table (2) show that the fruit yield/ plant and fruit yield/ fed were considerably enhanced as a result of applying liquorice, chitosan or dry yeast treatments relative to the control in the two experimental seasons. Moreover, plants sprayed with chitosan resulted in the highest yield compared to those sprayed with liquorice or dry yeast treatments. The fruit yield/fed was increased by 134.20 and 145.65 % over control when chitosan treatment was applied in both seasons, respectively. The enhanced anise fruit yield due to liquorice application may be attributed to the improved mobilization of growth-linked metabolites dissolved substances such as mineral nutrients, soluble sugars, antioxidants, and amino acids in liquorice that positively reflect on growth enhancing and consequently fruit production. Similar trend was previously reported (Desoky *et al.*, 2019). These results are in agreement with those obtained by Abd El-Azim *et al*. (2017) who found that liquorice water extract not only improved the growth but also the fruit yield of coriander. Increasing the yield due to chitosan application may be ascribed to the effective role of chitosan in improving the growth and consequently reflected in increasing the fruit yield. Similar findings were reported by Malekpoor *et al*. (2016). Furthermore, chitosan was found to enhance the secondary metabolites accumulation in herbs and medicinal plants (Zhao *et al*., 2005) through activating the genes that motivate the biosynthetic pathways of secondary metabolites (Lei *et al*., 2011). The current results are in agreement to those obtained by Bistgani *et al*. (2017) in basil. Stimulating the growth by dry yeast may be a logic reason to improve the fruit yield as well which in accordance with the results of Kassem (2013). Such increase in yield due to dry yeast treatment was documented by several researchers (El-Sayed *et al*., 2002 and Ezz El-Din and Hendawy, 2010).

Table 2. Effects of some biostimulants on fruit yield and volatile oil content of anise plant during 2017/2018 and 2018/2019 seasons.

Biostimulants	Fruit yield/plant (g)	Fruit yield/fed (kg)	Volatile oil (%)	Volatile oil yield/plant (mL)	Volatile oil yield/fed (L)
			2017/2018 season		
Control (Tap water)	6.52d	144.89d	2.15d	0.140d	3.12d
Liquorice (20 g/L)	11.33c	251.78c	2.39c	0.271c	6.02c
Chitosan (400 mg/L)	15.27a	339.33a	2.76b	0.421a	9.37a
Dry yeast (6 g/L)	13.87b	308.22b	2.64a	0.366b	8.14b
			2018/2019 season		
Control (Tap water)	6.44d	143.11d	2.13d	0.137d	3.05d
Liquorice (20 g/L)	10.72c	238.22c	2.31c	0.248c	5.50c
Chitosan (400 mg/L)	15.82a	351.55a	2.67a	0.422a	9.39a
Dry yeast (6 g/L)	14.64b	325.33b	2.61b	0.382b	8.49b

Means had different letters in each season are significantly differ from each other according to Duncan's multiple range test at 5 % ($P \leq 0.05$).

3. Volatile oil content and composition

The volatile oil percentage in anise fruits and volatile oil yield were significantly increased by the various biostimulants application. Plants sprayed with liquorice, chitosan or active dry yeast recorded significantly higher fruit as well as volatile oil yield compared to the control during the two experimental seasons (Table 2). The highest volatile oil percentage and yield were obtained by applying chitosan treatment. Chitosan application was superior to liquorice or active dry yeast treatments in this respect as increased by 200.65 and 207.93 % over the control in both seasons, respectively. The GC MS analysis of volatile oil was presented in Table (3). Nine components were defined in anise volatile oil and these components were α -pinene, β-pinene, Di-pentene, limonene, linalool, methyl chavicol, anethole, anis-aldehyde and carvone. The main constituents of volatile oil were anethole, carvone, anis-aldehyde and methyl chavicol. The anethole component recorded the highest percentage in volatile oil. Interestingly, liquorice, chitosan or active dry yeast applications improved the volatile oil components relative to untreated plants. Plants sprayed with dry yeast resulted in the highest percentage of anethole however, chitosan application gave the highest percentages of carvone, anis-aldehyde and methyl chavicol.

Enhancing the volatile oil content due to liquorice application may be due to its role in motivating the photosynthesis and elevating the sink capacity fulfilled during supply of photo-assimilates from leaves and consequently improved the secondary metabolites accumulation (Thomas and Howarth, 2000). Elevating the volatile oil content as a result of chitosan treatment may be due to that chitosan may act as a potent inducer for enhancing the biosynthesis of secondary metabolites. Previous reports also indicated that chitosan increased the essential oil content (Yin *et al*., 2011, on oregano and Bistgani *et al*., 2017 on thyme). Furthermore, accumulating more volatile oil due to dry yeast application which is in agreement with current results was previously reported by Sharaf-Eldin *et al*. (2008) on lemon balm; Ezz El-Din and Hendawy (2010) on *Borago officinalis*.

4. Photosynthetic pigments, carbohydrate content and nutrient elements

The results of chemical analysis of anise herb are presented in Table (4). It could be noticed that the total chlorophyll and carotenoids contents were enhanced as a result of applying the various biostimulants compared to the control in both seasons of the study. Additionally, the total carbohydrates as well as N, P and K percentages were also increased due to spraying with the investigated biostimulants relative to untreated plants. The highest total chlorophyll and carotenoids contents were observed by applying chitosan treatment followed by dry yeast application without significant differences in between. Otherwise, plants sprayed with chitosan recorded the highest total carbohydrates as well as N, P and K percentages in both the experimental seasons relative to either liquorice or dry yeast treated plants. The lowest values of all chemical traits investigated were recorded in control plants in both seasons. Increasing the mineral nutrients could be ascribed to the presence of these elements in liquorice extract and they are important for plant growth and development and consequently the uptake of these elements may increase as noticed in our results. In this respect, Thanaa *et al*. (2016) reported that liquorice extract application may increase the endogenous hormones like GA_3 in treated plants which improve the metabolic processes and hence increase the nutrient content in tissues. Increasing photosynthetic pigments and carbohydrates in the current study are in accordance with the results of Nasser *et al.* (2014) and Hassan and Abd El-Samee (2015).

Table 3. Effects of some biostimulants on volatile oil constituents of anise fruits during 2017/2018 and 2018/2019 seasons.

Biostimulants	Volatile oil constituents (%)								
	α -Pinene	β -Pinene	Di-pentene	Limonene	Linalool	Methyl chavicol	Anethole	Anis-aldehyde	Carvone
Control (Tap water)	0.157	0.172	0.213	0.377	0.613	2.11	65.64	2.18	3.37
Liquorice (20 g/L)	0.159	0.170	0.220	0.381	0.611	2.17	68.37	2.29	3.92
Chitosan (400 mg/L)	0.166	0.181	0.225	0.379	0.624	2.33	73.55	2.67	5.12

Dry yeast (6 g/L)	0.169	0.183	0.228	0.384	0.628	2.30	73.92	2.37	5.17

The GC-MS was investigated in the second season of the experiment.

It was reported that mineral nutrients maintain photosynthetic machinery through activation of the PSII reaction centers and regeneration of ribulose-1,5-bisphosphate which maximize photosynthesis and elevating the sink capacity fulfilled during supply of photo-assimilates from leaves (Thomas and Howarth, 2000). Therefore, the photosynthetic pigments and total carbohydrates were increased. Such increment in investigated chemical analysis due to liquorice extract was observed by Desoky *et al.* (2019). Our results showed that the increase of chlorophyll content by the various investigated biostimulants reflected on enhancing growth and yield attributes that might be ascribed to more assimilations correlated with nutrient elements and GA_3 (Alireza *et al.,* 2014). Improving the photosynthetic pigments due to chitosan treatment supports the findings of El-Tantawy (2009) who reported chitosan application increased photosynthetic pigments thereby increasing the photosynthesis. Otherwise, Khedr and Farid (2000) reported that dry yeast show ability in stimulation of endogenous hormones such as GA_3 and IAA may increase the photosunthetic pigments and carbohydrates as well as motivating the nutrients uptake.

Table 4. Effects of some biostimulants on photosynthetic pigments, total carbohydrates and nutrient elements of anise plant during 2017/2018 and 2018/2019 seasons.

Biostimulants*	Total chlorophyll (mg/g FW)	Carotenoids (mg/g FW)	Carbohyd-rates (%)	N (%)	P (%)	K (%)
			2017/2018 season			
Control (Tap water)	0.89c	0.27c	8.89d	1.22d	0.22d	1.77d
Liquorice (20 g/L)	1.18b	0.33b	11.67c	1.78c	0.29c	2.11c
Chitosan (400 mg/L)	1.32a	0.42a	14.24a	2.12a	0.31a	2.43a
Dry yeast (6 g/L)	1.31a	0.41a	12.58b	2.04b	0.29b	2.35b
			2018/2019 season			
Control (Tap water)	0.91c	0.28c	8.92d	1.25d	0.23d	1.74d
Liquorice (20 g/L)	1.15b	0.34b	11.78c	1.77c	0.28c	2.31c
Chitosan (400 mg/L)	1.30a	0.45a	14.77a	2.34a	0.33a	2.53a

Dry yeast (6 g/L)	1.29a	0.44a	12.86b	2.11b	0.32b	2.44b

Means had different letters in each season are significantly differ from each other according to Duncan's multiple range test at 5 % ($P \le 0.05$).

Conclusion

Foliar spraying with liquorice, chitosan or dry yeast significantly enhanced the growth character and fruit yield components of anise. Application of those biostimulants not only enhanced the volatile oil yield but also increased the main components in volatile oil. Moreover, photosynthetic pigments, total carbohydrates and N, P, K percentages were considerably increased as well. To stimulate the growth and increase the quantity and quality of fruit and volatile oil yield of anise, foliar spraying with chitosan at 400 mg/L followed by active dry yeast at 6 g/L was suggested. The spraying is suggested to be three times at one month interval after one month from thinning.

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تأثير بعض المنشطات الحيوية علي النمو والمحصول ومحتوي الزيت الطيار في نبات الينسون

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الملخص العربى

أجريت هذه التجربة لدراسة تأثير المعاملة بكل من مستخلص جذور نبات العرقسوس والشيتوسان والخميرة كمنشطات حيوية علي النمو والمحصول ومحتوي الزيت الطيار في نبات الينسون. وقد تم رش النباتات بمستخلص جذور نبات العرقسوس بمعدل 20 جم/لتر بينما استخدم الشيتوسان بتركيز 400 مجم/لتر والخميرة بتركيز 6 جم/لتر. وتم رش نباتات الكنترول بماء الصنبور.

وأوضحت نتائج هذه التجربة أن الرش بأي من هذه المنشطات أدي الي تحسين معنوي في صفات النمو الخضري مقارنة بنباتات الكنترول في كل من موسمي التجربة. وكذلك فان محصول الثمار للنبات والفدان ازداد معنويا بالرش باستخدام أي من هذه المنشطات مقارنة بالنباتات الغير معاملة. ومن الجدير بالذكر أن المعاملة بمستخلص جذور نبات العرقسوس والشيتوسان والخميرة لم تؤدي فقط الي تحسين النسبة المئوية للزيت الطيار وكذلك محصول الزيت ولكنها أدت أيضا الي تحسين مكونات الزيت الطيار الرئيسية. كما أوضحت النتائج أن صبغات التمثيل الضوئي والكربوهيدرات والنسبة المئوية للنيتروجين والفوسفور والبوتاسيوم قد ازدادت بمعاملة الرش بأي من هذه المنشطات الحيوية سالفة الذكر. وبصفة عامة فان أكثر هذه المنشطات الحيوية تأثيرا كان الرش بالشيتوسان ثم الرش بالخميرة في كل من موسمي التجربة. لتنشيط نمو نبات الينسون وزيادة محصول الثمار والزيت الطيار من حيث الكمية والجودة يوصي باستخدام معاملة الرش بالشيتوسان بتركيز 400 مجم/لتر علي أن يتم الرش ثلاث مرات علي فترات شهرية تبدأ بعد مرور شهر من عملية الخف.

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