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Effect of Organic Manure and Bio-fertilizers on Productivity and Quality of Cumin (*Cuminum cyminum*, L.) Plant Grown in Calcareous Sandy Soil

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Abstract

An experiment was conducted at the experimental farm of Agricultural Research station, Arab-El-Awamer, Assiut Governorate, Egypt, to evaluate the effect of bio-fertilizers in combination with different rates of organic manure on growth, yield and oil percentage of cumin (*Cuminum cyminum*) in the growing seasons of 2012/2013 and 2013/2014.The studied treatments included four rates of cattle manure as organic fertilizers; 0 (control), 12.5, 25, 37.5 m^3/fed. were considered as main plots and four treatments of bio-fertilizers., 0 (without inculation), *Azotobacter chroococcum(Az), Bacillus megatherium (Bm) and Bacillus circulans* (Bc) as sub plots. The obtained results indicated that the studied characters were significantly increased with increasing the rate of organic fertilizer.The highest yield of fruits and oil percentage were obtained at 37.5 m^3/fed. However, the combination between organic treatment and bio-fertilizers was significantly higher than organic manure alone, where the inoculation with bio-fertilizer Az or Bm combined with 37.5 m3/fed. of cattle manure improved the most growth parameters, yield and oil percentage compared to other treatments. The fertilization of plants with cattle manure 37.5 m^3/fed.+Bc resulted in the highest of many components e.g., α-Thujene β-Pinene P-Cymene Phyllandrene and Cumin aldehyde recorded 1.664, 5.429, 11.489, 19.661and 32.623% respectevely So, applying 37.5 m^3/fed.cattle manure combined with *Azotobacter* or *Bacillus megatherium* as bio-fertilizer significantly improved quantitative and qualitative yield of cumin and produced high quality product for human health.

Keywords: Cumin, cattle manure, bio-fertilizer, *Azotobacter* and essential oil

Introduction

Medicinal and aromatic plants have the economic importance because of the continuous and increasing demand for their products by local and international markets (Khalid, 2006). Recently, the production of chemical- free medicinal and aromatic plants has been the focus of interest of many researchers and producers in order to ensure the quality and safety, not only for human, but also for the environment (Abo-Baker and Mostafa, 2011). Cumin (*Cuminum cyminum* L.) a member of family Apiaceae, is an annual plant which is originated from Egypt and East Mediterranean. It is regarded as the most important medical seed spice in Egypt. It is a small and annual umbelliferous plant that has a short growing season. Its aromatic seeds

have medical applications and plant essential oils have many applications such as changing the smell of some medicines equipping the product antiseptic, sterilization of surgical operation fiber, product of some veterinary and agricultural medicines and industry perfumery (Yilmaz and Arsian, 1991). More recently, there has been a revival of interest in environmentally friendly, sustainable and organic agricultural practices. There are some supporting studies that addition of organic material affects some properties of the soil, organic material improves soil structure and aggregate stability, as well as its moisture retention capacity (Bhattacharyya *et al*., 2008). Manure is typically applied to the soil at rates designed to supply a crops nitrogen requirement (Evanylo *et al*., 2008). The use of organic fertilizers and cattle caused the increase in cumin seed biological performance and the number of umbrellas in the crucible (Sydnzhad and Rezvani, 2009). Saboor (2004) reported that manure application increases cumin yield. Bio-fertilizers differ from organic fertilizers in that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. The use of bio-fertilizers is known to improve plant growth through the supply of plant nutrients and may help to sustain environmental health and soil productivity (O, Connell, 1992). Research has demonstrated that using bio-fertilizer could reduce the need for chemical fertilizer and decrease its adverse effects on the environment (Karlidag *et al*., 2007). In addition, the use of bio-fertilizers on Egyptian soils has decreased the pH, which lead to increased availability of trace elements that enhance plant growth (Mahfouz and Sharaf- Eldin, 2007). *Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberllic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients and photosynthesis (Fayez *et al.,* 1985). Also the use of biological fertilizers containing the bacteria *Azotobacter* and *Azospirillum* in medicinal plants (salvia and thyme) caused a significant increase in plant growth (Youssef *et al*., 2004). Yousry *et al*. (1978) found that inoculation of *Pisum sativum* plants with *Bacillus megatherium* increased plant dry matter by 10.9%, while the combined application of *Bacillus megatherium* and P-fertilizer increased plant dry matter by 19.7%. Regarding the necessity of organic and bio-fertilization in medical plants as well as the economic and medical importance of cumin, this experiment was conducted to evaluate responses of cumin to organic and bio-fertilizer in calcareous sandy soil.

Materials and Methods

A field experiment was carried out during two successive seasons (2012-2013 and 2013-2014) at the experimental farm of Agricultural Research station, Arab-El-Awamer, Assiut Governorate, Egypt, to study the effect of bio-fertilization in combination with organic manure on growth parameters, fruit and oil yield, oil percentage and chemical constituents of the oil of cumin plants. The soil physical and chemical character-

isitics of the experimental field are shown in Table (1) which was done according to the methods described by Black *et al*. (1982) and Jackson (1973). Sowing was done in a linear pattern in November 1st in a split-plot arrangement based on a Randomized Complete Block Design (RCBD) with three replicates for each treatment. Each plot of 2*2m contains 3 rows 60cm apart and broadcast at both sides of the ridge with uniform seed rate of 12 kg/fed.. The seeds were planted, after germination, thinning was applied to the desired density. Studied treatments included four levels of cattle manure as organic fertilizers (0($org._0$), 12.5($org._1$), 25 ($org._2$), 37.5($org._3$) m^3/fed.) were considered as main plots and four treatments of bio-fertilizers 0 (without inoculation), *Azotobacter chroococcum* (Az) as N_2 fixing bacteria, *Bacillus megatherium* (Bm) as phosphate dissolving bacteria *and Bacillus circulans* (Bc) as potassium releasing, biofertilizers inocula were added at the rate of 600 gm/fed., each carrier inoculum contains 10^9cfu/gm, whereas biofertilizers inocula were obtained from Central lab. of Organic Agriculture). All treatments were added and mixed with soil at the time of planting. At the end of growing seasons, morphological and yield traits (Plant height (cm), number of branches /plant, number of umbels/plant, fruit dry weight (g)/plant were measured randomly in 10 plants from each plot, fruit yield (kg)/fed. was determined by harvesting $4m^2$ with regard to border effects), as well as oil percentage and oil yield litre/fed. were measured and averaged.

Table 1. The physical and chemical characteristics of representative composite soil sample from the field experimental site (Hashem *et al*., 2016)

Soil Properties	Values
Particle size distribution	
Sand (%)	89.9
Silt (%)	7.1
Clay (%)	3.0
Soil texture	Sandy
Field capacity (%)	10.9
Water saturation (%)	23.3
Total CaCO3 (g kg^{-1})	300
EC mmhos/cm soil water extract, 1:1	1.6
pH (1 : 1 water suspension)	8.46
Organic matter (g kg^{-1} soil)	2.4
Soluble cations(mmolc L^{-1}) :	
Ca^{++}	3.4
Mg^{++}	2.54
Na^{+}	9.1
K^{+}	0.96
Soluble anions (mmolc L^{-1}):	
CO_3^- HCO_3^-	8.7
Cl^-	6.1
SO_4^{--}	1.2
Total nitrogen (mg kg^{-1})	130
Available Phosphorus (mg kg^{-1})	10.75
Available potassium (mg kg^{-1})	54.6

Essential oil percentage of fruits was determined using 100g samples for each treatment. Distillation of the volatile oil was as described in the British Pharmacopoeia.

The volatile oil obtained was analyzed using Ds Chrom 6200 Gas Chromatograph equipped with a

flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follows:- The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyl (equiv.) polysill-phenylene-siloxane 30m x 0.25mm ID x 0.25 film- Temperature program ramp increase with a rate of 10^{o}C / min from 70^{o} to 200^{o}C. Flow rates of gases were nitrogen at 1ml/min, hydrogen at 30 ml /min and330ml/min for air. Detector and injector temperatures were 300^{o}C and 250^{o} C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil.

Data were statistically analyzed according to Snedecor and Cochran, (1973) and Least Significant Difference (LSD) test was applied at 5% probability level to compare the means of various treatments according to Steel and Torrie (1982).

Results and Discussion

Effect of cattle manure:

Data in Table (2) showed that increasing amount of cattle manure from 12.5 to 25m^3/fed., significantly increased plant height, number of branches /plant, number of umbels/ Plant, fruit dry weight (g)/plant and kg/fed. in both seasons. Fruits yield (kg)/ fed. significantly increased from 361.67 to 432.40 kg by raising cattle manure from 12.5 to 37.5 m^3/fed. in the first season. These results are in agreement with those of Mallanagouda (1995) on coriander, Seghatoleslami (2013) and Talaei *et al*. (2014) on fennel. The results showed that effect of different rates of cattle manure on essential oil percentage and oil yield L./fed. Figure (1) was also significant, while, variation trend of essence contents was positive relation with increasing of cattle manure recorded in the application of 0, 12.5, 25 and 37.5 m^3/fed. cattle manure with averages of 2.28, 2.32, 2.33 and 4.92% . These results were coinciding with those of the second season (Table 3), whatever the second season showed higher results than that of the first one may due to the improving effects of manure from the first season. In the same trend, Sydnzhad and Rezvani (2009) reported that use organic fertilizers and cattle caused the increase in performance of cumin seed, the number of seed in umbrella and bushes. Manure application improves the soil structure and soil moisture content, provides plant with essential elements, increases growth, number of umbrella per plant and biological yield and finally led to increase seed yield (Ahmadian *et al*., 2011).

Effect of bio-fertilizers:

Data in Tables 2 & 3 showed that fertilizing cumin plants using different strains of bio-fertilizers, Az, Bc and Bm increased plant height, number of branches and oil percentage compared with control treatment (without inoculation) in the two seasons. There were no significant differences between fruit yield of control and bio-fertilizer strains Bc and Bm, wherever the largest amount of growth and oil yield L./fed. occurred with strain *Azotobacter chroococcum* in both seasons (Figure2). These results are in agreement with Fayez *et al*.,(1985) and Mahfouz and Sharaf-Eldin,(2007), they reported that these results may be due to the role of

Azotobacter in nitrogen fixation, in addition, it provides growth promoting substances such as indole acetic acid and Gibberellins. Tehlan *et al*., (2004) reported that plant growth and seed yield of fennel varied according to the strain applied. The maximum umbel number per plant, biological yield and seed yield of anise were obtained using *Bacillus circulans*, the phosphate solubilizing bacterium (Mohammed *et al*. 2012).

Effect of combination:

The results in Tables 2 &3 showed that combination between cattle manure and strains of *Azotobacter* and *Bacillus m* had augmented number of umbels/ plant, fruit yield (kg)/fed. and oil percentage compared with control or cattle manure alone. The highest values of the most characters have been recorded with applying cattle manure at 37.5m^3/fed.+Az, as well as, cattle manure 37.5m^3/fed. +Bm in the two seasons. These results showed variation was trend to positive relation with increasing of cattle manure and recorded the maximum fruit yield and essential oil percent (444.89, 5.82% respectively by 37.5m^3/fed.+Az) Table3. Gomaa and Mohamed (2007) studied the combined effect of organic and biofertilizers on guar plants, they found that the greatest forage yield per feddan produced from 20 ton FYM+ *Rhizobium*+ yeast(40L/fed.). Congruently , the results obtained by Valadabadi and Farahani (2013) showed that application of *Azotobacter* and animal manure increased essential oil, harvest index and productivity effort of black cumin.

Essential oil components

Analysis of essential oil samples of plants receiving certain fertilization treatments (0 (without any fertilizers), *Azotobacter* (Az), cattle manure25 m^3/fed. ($org._2$), cattle manure 37.5 m^3/fed. ($org._3$), 37.5 m^3/fed.+ *Azotobacter* ($org._3$+Az), 37.5 m^3/fed.+ *Bacillus circulans* ($org._3$+Bc) and 37.5 m^3/fed.+ *Bacillus* m($org._3$+Bm)) was illustrated in Table 4 as the main ten components. The highest total percentage of the main components (83.861%) resulted from treating the plants with cattle manure 37.5 m^3/fed.+Bc. Fertilization of plants with manure 37.5 m^3/fed.+Bc resulted in highest of many components compared to control and all other treatments while the same treatment gave the highest compound of α-Thujene, β-Pinene, P-Cymene, Phyllandrene and Cumin aldehyde recorded 1.664, 5.429, 11.489, 19.661 and 32.623% respectively. Cumin aldehyde was identified in all treatments.

Table 2. Growth, yield and oil content of cumin as influenced by different rates of organic with/without bio-fertilizers during the season of 2012/2013

Treatments		Plant height (cm)	No. of branches /plant	No. of umbels/ plant	Fruit dry weight g/plant	Fruit yield kg/fed.	Oil %
$Org._0$	0	21.20	5.4	14.87	1.45	337.88	1.91
	Az	22.03	6.53	15.10	1.43	345.85	2.43
	Bc	22.33	6.33	15.13	1.41	339.06	2.29
	Bm	22.63	6.07	14.90	1.39	339.90	2.48
Mean		22.12	6.083	15.00	1.42	340.67	2.28
$Org._1$	0	24.67	6.53	16.4	1.61	356.24	2.05
	Az	25.40	7.27	17.83	1.61	362.78	2.29
	Bc	24.90	6.63	17.13	1.62	360.68	2.77
	Bm	26.03	7.97	17.97	1.63	366.98	2.17
Mean		25.25	7.10	17.33	1.62	361.67	2.32
$Org._2$	0	27.30	7.33	18.43	1.75	384.88	2.21
	Az	27.43	8.37	19.97	1.87	413.08	2.39
	Bc	27.33	7.20	18.30	1.66	403.56	2.25
	Bm	26.00	7.53	19.77	1.86	417.42	2.45
Mean		27.02	7.61	19.12	1.79	404.74	2.33
$Org._3$	0	25.53	8.30	21.07	1.83	420.22	4.91
	Az	26.60	8.73	24.43	2.27	439.12	5.62
	Bc	25.77	8.40	23.43	2.08	425.40	4.35
	Bm	26.40	8.30	23.37	2.47	444.86	4.81
Mean		26.08	8.43	23.08	2.16	432.40	4.92
Means of	0	24.68	6.89	17.69	1.66	374.81	2.77
	Az	25.37	7.73	19.33	1.79	390.21	3.18
bioferilizer	Bc	25.08	7.14	18.49	1.69	382.18	2.92
	Bm	26.27	7.47	19.00	1.84	392.29	2.98
LSD 5%	A	1.02	0.15	0.31	0.07	4.55	0.13
	B	N.S	0.30	0.35	0.06	4.04	0.16
	AB	1.14	0.60	0.70	0.11	8.08	0.32
		(1.42)	(0.54)	(0.68)	(0.12)	(8.32)	(0.30)

Control (0): without inculation Az: *Azotobacter*; Bc: *Bacillus circulans*; Bm: *Bacillus megatherium* $Org._0$: without manure; $Org._1$:cattle manure 12.5 m^3/fed.; $Org._2$: cattle manure 25 m^3/fed.; $Org._3$: cattle Manure 37.5 m^3/fed. AB: revealed to compare the means for the same level (A); between parentheses is compare the means for different level of (A)

The highest percentage was obtained from 37.5m^3/fed.+Bc followed by cattle manure 25m^3/fed. Myrcene % contained was the highest with treatment of cattle manure 37.5 only. El-Ghadban *et al*., (2006) found that fennel responded to biofertilizer by increasing growth and oil yield and changing the chemical composition. Abdelaziz *et al*., (2007) on rosemary showed the highest oil yield was related to a combination of compost and microorganisms that cause increasing growth characteristics and chemical composition. Anwer *et al*., (2005) studied the effect of vermicompost on basil and observed that in addition to the oils of the plant to increase the amount of linalool and methyl kavykol was essence of.

Table 3. Growth, yield and oil content of cumin as influenced by different rates of organic with/without bio-fertilizers during the season of 2013/2014

Treatments		Plant height	No. of branches/ plant	No. of umbels/ plant	Fruit dry weight g/plant	Fruit yield (kg)/fed.	Oil %
Org.0	0	22.57	5.43	15.57	1.38	341.10	1.86
	Az	23.13	5.73	15.43	1.43	353.73	2.41
	Bc	24.60	6.30	15.67	1.40	350.12	2.34
	Bm	24.17	6.30	15.07	1.36	348.48	2.56
Mean		23.54	5.94	15.44	1.39	348.36	2.29
$Org._1$	0	25.17	6.77	16.87	1.60	361.84	2.40
	Az	25.63	7.43	18.60	1.79	363.69	2.36
	Bc	26.17	7.90	17.50	1.72	361.46	2.36
	Bm	24.67	7.50	18.30	1.70	367.76	2.27
Mean		25.41	7.4	17.82	1.70	363.69	2.35
$Org._2$	0	25.40	8.00	20.57	1.86	390.66	2.31
	Az	27.63	8.47	21.00	1.92	419.73	2.45
	Bc	26.37	7.30	19.30	1.81	410.04	2.24
	Bm	24.87	8.40	21.00	1.87	423.19	2.52
Mean		26.07	8.04	20.47	1.87	410.91	2.38
$Org._3$	0	28.97	8.80	23.13	2.05	426.17	4.27
	Az	27.50	9.60	25.47	2.60	444.89	5.82
	Bc	23.07	8.10	22.53	2.28	431.88	4.40
	Bm	26.33	9.63	24.30	2.41	449.73	4.82
Mean		26.47	9.03	23.86	2.34	438.17	4.83
Means of	0	25.53	7.25	19.04	1.72	379.94	2.71
	Az	25.97	7.81	20.13	1.94	395.51	3.26
bioferilizer	Bc	25.05	7.40	18.75	1.81	388.38	2.84
	Bm	25.01	7.96	19.67	1.84	397.29	3.04
LSD 5%	A	1.63	0.35	0.43	0.08	1.74	0.36
	B	N.S	0.34	0.53	0.06	2.06	0.30
	AB	3.04	0.68	1.06	0.11 (0.12)	4.12	0.60
		(3.08)	(0.68)	(1.02)		(3.96)	(0.63)

Control (0): without inculation Az: *Azotobacter*; Bc: *Bacillus circulans*; Bm: *Bacillus megatherium*, $Org._0$: without manure; $Org._1$: cattle manure 12.5 m^3/fed.; $Org._2$: cattle manure 25 m^3/fed.; $Org._3$: cattle Manure 37.5 m^3/fed. AB: revealed to compare the means for the same level (A); between parentheses is compare the means for different level of (A)

Table 4. Chemical composition of cumin essential oil as influenced by different rates of organic with/without bio-fertilizers during the season of 2013/2014

Treat. / Comp	Cont.	Az.	$Org._2$	$Org._3$	Az+ $Org._3$	Bc+ $Org._3$	Bm +$Org._3$
-Thujeneα	1.17819	0.85126	1.1381	0.54021	1.18331	1.66482	1.494
Pinene-β	5.04657	4.18726	3.86163	1.45407	3.7483	5.42962	4.88416
Myrcene	1.93522	1.73235	1.65566	9.18674	1.60578	1.92058	2.12728
Phyllandrene	0.44808	6.39758	8.4464	0.50722	0.39222	11.48919	0.38515
Uknown	9.47519	8.62695	4.59693	32.95761	9.89611	3.67621	10.42884
P-Cymene	16.77008	5.51621	16.22191	1.38988	16.40607	19.66138	17.6693
Terpinene-ɣ	14.924	1.07716	1.96646	1.49694	5.97166	1.66198	1.6499
Uknown	1.63224	30.12109	1.17798	3.49069	1.66691	1.04044	1.07999
Cumin aldehyde	0.93194	7.08006	31.51632	1.30291	1.00427	32.62334	29.0171
P-menth-i-en-7-al	22.72031	3.17891	5.41732	1.16414	28.57042	4.6934	5.67213
Total %	75.06182	68.76883	75.99871	53.49041	70.44505	83.86096	74.40785

Control (0): without any fertilizers Az:*Azotobacter*; Bc:*Bacillus circulans*; Bm:*Bacillus megatherium*

$Org._2$: cattle manure25 m^3/fed.; $Org._3$: cattle manure37.5 m^3/fed

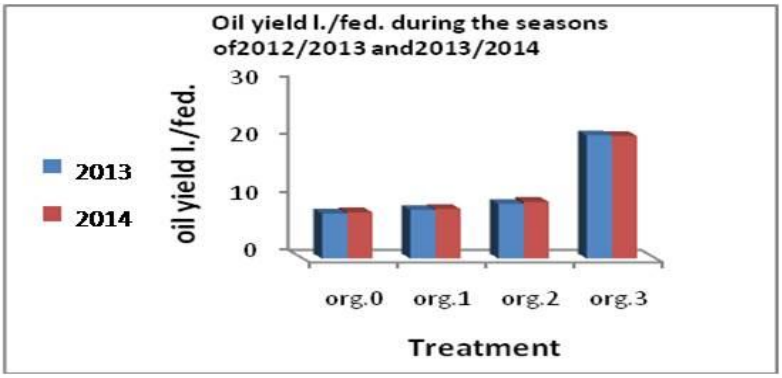

Figure 1: Effect of different rates of cattle manure on oil yield litre/fed. of cumin.

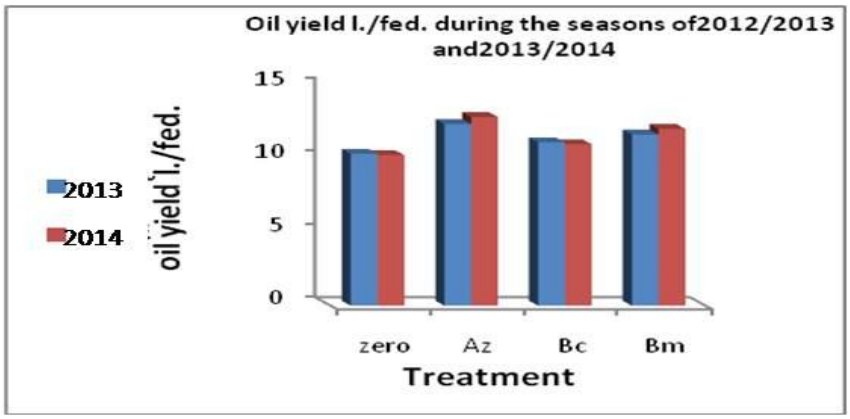

Figure 2: Effect of different strains of biofertilizer on oil yield litre/fed. of cumin

Conclusion

In general, based on analysis of results of this research, application of cattle manure improved yield and other criteria. It also appears that cattle manure at 37.5m^3/fed. combined with *Azotobacter* or *Bacillus megatherium* as bio-fertilizer significantly improved quantitative and qualitative yield of cumin (*Cuminum cyminum)* plant and produced high quality product for human health.

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تاثير السماد العضوى و المخصبات الحيوىة على انتاجية وجودة نبات الكمون فى الاراضى الجيرية الرملية

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الملخص

أجرى هذا البحث بمزرعة محطة البحوث الزراعية بعرب العوامر- أسيوط خلال الموسمين الزراعيين ٢٠١٣/٢٠١٢ و٢٠١٤/٢٠١٣ لدراسة تأثير التسميد العضوى متمثلا فى سماد الماشية بثلاثة معدلات مختلفة (١٢,٥ و ٢٥ و٣٧,٥م^{٣}/للفدان) والتسميد الحيوى متمثلا فى ثلاثة انواع من البكتيريا هي

Azotobacter chroococcum (Az), Bacillus circulans (Bc) *and Bacillus megatherium (Bm)*

وذلك على النمو ومحصول الثمار ونسبة الزيت الطيار وكذلك مكونات الزيت لنبات الكمون بهدف التوسع فى زراعته فى الاراضى الرملية. حيث تم اخذ البيانات اللازمة وحللت احصائيا وقد وجد الاتى:

كان لإضافة السماد العضوى بصفة عامة تأثيرا واضحا على نمو النبات وانتاج الثمار وكذلك الزيت مقارنة بالكنترول.

إضافة اي من معدلات سماد الماشية المتحلل وهى ١٢,٥ و ٢٥ و٣٧,٥م^{٣}/للفدان حدثت زيادة معنوية فى عدد الفروع وعدد النورات الخيمية ووزن الثمار بالنبات وكذلك إنتاج الفدان من الثمار الجافة والنسبة المئوية للزيت وانتاج الزيت للفدان.

أوضحت النتائج ايضا ان زيادة المحصول من الثمار ونسبة الزيت تتناسب طرديا مع زيادة المعدل المضاف من السماد حيث سجل المعدل ٣٧,٥ م^{٣}/للفدان أعلي النتائج فى موسمي الزراعة.

اظهرت النتائج ان هناك زيادة معنوية فى النمو والانتاجية وكذلك نسبة الزيت لنبات الكمون عند استخدام السماد العضوى والحيوى معا مقارنة باستخدام كل منهما على حده أو مقارنة بالكنترول.

كانت أفضل المعاملات المستخدمة هى إستعمال ٣٧ م^{٣} من سماد الماشية المتحلل مضافا اليه السماد الحيوى (جنس Bacillus *megatherium* أو Azotobacter) حيث سجلت افضل النتائج فى الصفات الخضرية ومحصول الثمار ونسبة الزيت ومكوناته.