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EFFECT OF SOME CLEAN AGRICULTURAL TREATMENTS ON FRUIT YIELD, ACTIVE INGREDIENTS AND CHEMICAL COMPOSITION OF *Ammi visnaga*, L.

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ABSTRACT

A field experiment was carried out at the Experimental Farm. of Fac. Agric. Minia Univ. during two seasons (2011/2012 and 2012/2013) to study the effect of compost (0, 5, 7.5 and 10 ton/fed.) and eight biostimulant treatments (E.M., S.A., vit. E, E.M. + vit. E, E.M. + S.A., S.A.+ vit. E, E.M. + S.A. + vit. E and control) on *Ammi visnaga*, L. plants. The data showed that using 10 ton/fed. compost significantly increased fruit yield /plant, khellin and visnagin contents and chemical composition (N, P and K %) comparing with other treatments. Treating plants with E.M., S.A., vit. E and all possible mixtures significantly increased fruit yield, active ingredients and percentages of N, P and K comparing with control treatment. The best treatments were E.M. + S.A. + vit. E following by E.M. + vit. E in this concern. It may be recommended to use compost at the level of 10 ton/fed. and treating the plants with the triple a mixture (E.M. + S.A. + vit. E) or the dual mixture (E.M. + vit. E) to give the highest values of fruit yield and active ingredients (khellin and visnagin) of *Ammi visnaga* plants.

INTRODUCTION

Khilla Baladi (*Ammi visnaga*, L.) is one of the important Pharmaceutical plants of the Apiaceae (Umbelliferae). The fruits contain two main chromones, khellin and visnagin, they are used as diuretic and antispasmodic in case of urethral stones (Buckingham, 1994).

Improving fruit yield and chemical constituents of *A. visnaga* can be achieved through better cultural practices, the major practices, which must be utilized are organic, biofertilization and some stimulants such as salicylic acid (S.A.) and vitamins.

The role of organic fertilization on increasing fruit yield and active ingredient was reported by Younis *et al.* (2004) and Kenawy (2010) on *A. visnaga*, Abdou *et al.* (2009a) and Ebrahimi *et al.* (2010) on borage, Raja and Veerakumari (2013) on *Withania somnifera* and Kurt and Ayan (2014) on tobacco plants.

Effective microorganisms (E.M.) was found to have stimulating effect on yield and active ingredient production as reported by Abdou *et al.* (2009a and 2009b) on borage and guar plants, respectively and Ali (2013) on *Calendula officinalis*.

Many authors found that α-tocopherol (vit. E) (as stimulant and antioxidant substance) had positive effect on yield and active ingredient of many plants such as flax (El-Lethy *et al.*, 2010), *Calendula officinalis* (Soltani *et al.*, 2012), roselle (Hassan, 2013), guar (Abdou *et al*, 2013b) and caraway (Botros, 2013).

S.A. treatment increased plant growth, yield production, as well as, some chemical constituents. Talaat *et al.* (2014) on *A. visnaga* found that application salicylic acid at 20 mg/l. increased fruit yield/plant, yield of essential oil and the useful component such as linalool, as well as, increased khellin content. Similar results were obtained by Zangeneh *et al.* (2010) on *Datura metal*, Tariq Aftab *et al.* (2010) on *Artemisia annua* and Abdou *et al.* (2012c) on moghat plants.

Therefore, the present study was carried out to investigate the effect of compost, E.M. and some stimulant substances (S.A. and vit. E), as well as, their interaction on fruit yield, active ingredients and chemical constituents of *A. visnaga*.

MATERIALS AND METHODS

A field experiment was carried out during the two successive seasons of 2011/2012 and 2012/2013 at the Floriculture, Nursery and Laboratory of Pharmacy, Fac. of Agric. and Fac. of Pharmacy, Minia Univ. Minia, Egypt.

1- Plant material:

The fruits of *A. visnaga* were obtained from Medicinal and Aromatic Plants Dept., Horticulture Research Institute Giza, Egypt.

2- Experimental soil:

The physical and chemical analysis of the used soil in both seasons are shown in (Table **1**).

3- Layout of the experiment:

The experiment was arranged in a randomized complete block design in a split plot design with three replicates. The main plots (A) included four levels of compost (0, 5, 7.5 and 10 ton/fed.), while eight treatments (control, (E.M. at 50 ml/plant), (S.A. at 150 ppm), (vit. E at 20 ppm), E.M. + S.A., E.M. + vit. E and E.M. + S.A.

+ vit. E) occupied the sub-plots (B). Therefore, the interaction treatments (A×B) were 32 treatment.

Table 1: The physical and chemical analysis of the used soil in both seasons.

Soil Character	Value		Soil Character		Value	
	2012	2013			2012	2013
Sand %	28.30	28.78	Available P %		15.13	15.57
Silt %	29.99	30.55	Exch. K^+ mg/100 g		2.12	2.81
Clay %	41.71	40.67	Exch. Ca^{++} mg/100 g		31.76	31.14
Soil type	Clay loam	Clay loam	Exch. Na^+ mg/100 g		2.42	2.50
Organic matter %	1.54	1.62	DTPA Ext. ppm	Fe	8.51	8.21
Ca CO_3 %	1.58	1.54		Cu	2.07	2.01
pH (1: 2.5)	7.84	7.71		Zn	2.76	2.89
E.C. (m mhos / cm)	1.08	1.04		Mn	8.24	8.12
Total N %	0.07	0.08				

4- Experimental procedures:

Fruits of *A. visnaga* plant were sown on October, 20th in the first and second seasons. The experimental unit (plot) was 2.0×2.1 m. and containing three rows spaced at 70 cm. Fruits were sown on hills spaced at 40 cm. After six weeks from sowing, the growing seedlings were thinned to one plant/hill.

The used compost (called compost El-Neel) was obtained from the Egyptian Co. for Solid Waste Utilization, New Minia City. Its composition was shown in (Table **2**). Compost was added during preparing the soil to cultivation in both seasons.

Fresh and active E.M. was applied twice to the soil beside the plant at 50 ml/hill (1 ml contains 107 cells of bacteria). The first dose was added after seven weeks from planting date, while the second one was applied after one month from the first one in both seasons, and then plants were irrigated immediately.

Each of S.A. and vit. E were applied, twice by hand sprayer. The first one was added after seven weeks from planting date and one month interval.

The plants were harvested on the first week of June in both seasons and fruits yield/plant were recorded. The concentrations of khellin and visnagin in fruit extract were calculated according to Abdel-Salam *et al.* (1985). Total chlorophylls in the fresh leaves were determined according to Moran (1982). Nitrogen % (N.) was determined according to the modified Microkjeldahle method as described by Wilde *et al.* (1985). Phosphorus % (P.) was determined colorimetrically by the spectrophotometer at wavelength of 650 µm according to the method of Chapman and Pratt (1975), and potassium % (K.) was determined using flame-photometry

method according to Cottenie *et al.* (1982).

Table 2: Physical and chemical properties of the used compost

Properties	Value	Properties	Value
Dry weight of 1 m^3	450 kg	NaCl %	1.1-1.75
Fresh weight of 1 m^3	650-700 kg	Total P %	0.5-0.75
Moisture (%)	25-30	Total K %	0.8-1.0
pH (1:10)	7.5-8	Fe ppm	150-200
E.C. (m mhose/cm)	2-4	Mn ppm	25.56
Total N. %	1-1.4	Cu ppm	75-150
Org. matter %	32-34	Zn ppm	150-225
Org. carbon %	18.5-19.7		
C/N ratio	18.5-14.1		

The statistical analysis of data was carried out according to MSTAT-C (Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments Version 4.0, Michigan State Univ., U.S.A.). L.S.D test at 0.05 was used to compare the means of treatments.

RESULTS AND DISCUSSION

1- Effect of compost. E.M., S.A., vit. E and their four mixed treatments on fruit yield of A. visnaga*:*

Data presented in (Table **3**) showed that fruit yield/plant significantly increased by increasing the compost level in both seasons. Similar results were obtained by Younis *et al.* (2004) and Kenawy (2010) on *A. visnaga*. As for E.M. and/or some stimulant substances, data also showed that fruit yield/plant was significantly increased by using all seven treatments comparing with the control treatment. The best treatments, which gave the heaviest fruit yield/plant were a mixture of E.M. + S.A. + vit. E, followed by E.M. + vit. E.

The beneficial effects of biofertilizers on seed yield were obtained by El-Sawy *et al.* (1998); Attia (2001), Nofal *et al.* (2001) and Kenawy (2010) on *A. visnaga* and Abdou *et al.* (2009b) on guar plants who concluded that seed yield/plant and /fed. were augmented by inoculation E.M. In agreement with our results concerning S.A. were those reported by Talaat *et al.* (2014) on *A. visnaga*. Also, our results are harmony with the findings of Hassan (2013) on roselle, Botros (2013) on caraway and Abdou *et al.* (2013b) on guar concerning desirable effect of vit. E.

The interaction showed significant differences between compost and biofertilization treatments. The heaviest fruit

yield/plant was due to fertilizing plants with high or medium level of compost in combination with E.M. + S.A. + vit. E or E.M. + vit. E.

2- Effect of compost, E.M., S.A., vit. E and their four mixed treatments on khellin and visnagin contents in fruits of* A. visnaga*:

Data presented in (Table **3**) indicated that all levels used of compost significantly increased khellin and visnagin contents as compared with control in both seasons. The high level of compost (10 ton/fed.) recorded the highest contents of khellin and visnagin.

Similar results were obtained by Younis *et al.* (2004) and Kenawy (2010) on *A. visnaga*.

Also, (Table **3**) cleared that all seven treatments of E.M. and/or the two stimulant substances (S.A. and vit. E) significantly increased the contents of khellin and visnagin facing the control treatment. The highest contents of khellin and visnagin were given due to the application of the triple treatment E.M. + S.A. + vit. E.

The beneficial effects of biofertilizers on active ingredients were obtained by El-Sawy *et al.* (1998); Attia (2001); Nofal *et al.* (2001) and Kenawy (2010) on *A. visnaga*. Several investigators pointed out that vit. E was much effective in improving active ingredients of various plants, such as Hassan (2013) on roselle, Abdou *et al.* (2013a) on caraway and Shehata (2013) on guar plant. Moreover, Talaat *et al.* (2014) on *A. visnaga* found that S.A. treatment increased essential oil and khellin content.

The interaction between main and sub plots (A×B) was significant and the highest values were obtained at the high level of compost (10 ton/fed.) in combination with a triple treatments of E.M. + S.A. + vit. E.

***3- Effect of compost, E.M., S.A., vit. E and their four mixed treatments on the chemical composition of* A. visnaga:**

Data in (Table **4**) indicated that using compost significantly increased N., P. and K. % by increasing the applied level up to 10 ton/fed. These results were in agreement with those obtained by Ali *et al.* (2010) on anise, Abdou *et al.* (2009a) and Ebrahimi *et al.* (2010) on borage and Abdou *et al.* (2012b and 2012c) on sage and moghat plants, respectively.

In regard to, E.M., S.A., vit. E and their mixed treatments, all used seven treatments significantly increased N., P. and K. % in the dry herb of *A. visnaga* in comparison with control. The best results for N. % were obtained due to the use of E.M. + S.A. + vit. E, followed by E.M. + vit. E then E.M. + S.A. treatments without significant differences among them in both seasons. While, the highest percentages of P. and K. were recorded with the treatments of E.M. + S.A. + vit. E and E.M. + vit. E without significant differences between them in both seasons. Similar results were obtained by Abdou *et al.* (2009a and

2009b) on borage and guar plants respectively and Ali (2013) on *Calendula officinalis* concerning the effect of E.M. While Ismail (2008) on black cumin and Abdou *et al.* (2012a, 2013a and 2013b) on mint, caraway and guar plants, respectively regarding the effects of vit E.. While Shala (2012) and Botros (2013) on caraway concerning the effects of S.A.

The interaction between main and sub plots (A×B) was significant for N. and P. % in both seasons. The highest values of N. % were obtained by compost at 10 ton/fed. (in both seasons) or 7.5 ton/fed. compost (in the first season) in combination with E.M. + S.A. + vit. E, E.M. + vit. E and E.M. + S.A. While, the best interaction treatments for P. % were 10 ton/fed. compost × E.M + S.A. + vit. E or E.M. + vit. E.

Compost enhanced fruit yield, active ingredients and elements of N., P. and K. % because it is known to improve soil physical and biological properties i.e. water retention capacity, drainage, pH, better availability of soil micro-organism and reducing the negative impact of chemical based pesticides and fertilizers in the eco-systems (Zheljazkov and Warman, 2004).

E.M. contains useful organisms including photosynthetic bacteria, lactic acid bacteria, yeast and others. Ho and Hwan (2000) reported that E.M. is effective in enhancing soil properties, promoting yield and chemical composition of plants, as well as, augmenting tolerance.

The positive effects of S.A. may be attributed to its role in nutrient uptake, water relations, stomatal regulation, photosynthesis and growth (Khan *et al.* 2003 and Arfan *et al.*, 2007).

Vit. E is one of the most important components of cellular defens against oxidative injury and protect the plants against various environmental stresses (Fryer, 1992 and Hess, 1993). It may be recommended to use the compost at 10 ton/fed. and treated the plants with a mixture of E.M. + S.A. + vit. E to give the highest values of fruits yield and active ingredients.

Table (3): Effect of compost, E.M. and/or some stimulant substances treatments (S.A. and vit. E) and their four mixed treatments on fruit yield/plant, khellin % and visnagin % of *A. visnaga*, during 2011/2012 and 2012/2013 seasons.

Treatment	Compost levels (ton/fed.) (A)									
	1st season					2nd season				
	0	5	7.5	10	Mean (B)	0	5	7.5	10	Mean (B)
Fruit yield/plant (g)										
Control	25.32	26.71	35.31	35.90	30.81	30.39	33.34	34.23	46.07	36.01
E.M.	32.48	39.13	44.33	51.04	41.75	41.00	42.44	46.19	55.71	46.34
S.A. at 150 ppm	28.50	32.48	39.64	42.04	35.67	35.92	38.64	41.68	49.63	41.47
vit. E at 20 ppm	32.10	33.33	41.44	48.62	38.87	39.63	39.77	42.89	52.38	43.67
E.M. + S.A.	38.26	42.97	50.52	54.22	46.49	43.31	45.98	49.88	58.82	49.50
E.M. + Vit. E	40.11	43.99	52.47	56.13	48.18	43.44	49.71	54.20	59.62	51.74
S.A. + Vit. E	37.48	41.96	45.08	53.96	44.62	43.09	44.04	48.49	56.36	48.00
E.M. + S.A. + Vit. E	40.26	46.03	58.63	58.83	50.94	44.76	57.64	57.72	60.88	55.25
Mean (A)	34.31	38.33	45.93	50.09		40.19	43.95	46.91	54.93	
L.S.D. at 5 %	A: 4.00		B: 4.32		AB: 8.65	A: 3.75		B: 3.52		AB: 7.04
Khellin %										
Control	0.310	0.401	0.351	0.369	0.358	0.316	0.452	0.376	0.404	0.487
E.M.	0.358	0.430	0.413	0.454	0.414	0.361	0.462	0.438	0.496	0.539
S.A. at 150 ppm	0.414	0.431	0.504	0.529	0.469	0.408	0.431	0.525	0.558	0.580
vit. E at 20 ppm	0.599	0.604	0.690	0.770	0.666	0.699	0.705	0.826	0.939	0.892
E.M. + S.A.	0.469	0.470	0.563	0.749	0.563	0.480	0.481	0.602	0.843	0.702
E.M. + Vit. E	0.729	0.781	0.992	1.049	0.888	0.755	0.817	1.171	1.139	1.045
S.A. + Vit. E	0.632	0.699	0.773	0.808	0.728	0.639	0.719	0.807	0.850	0.854
E.M. + S.A. + Vit. E	0.628	0.972	1.079	1.881	1.140	0.691	1.069	1.187	2.070	1.354
Mean (A)	0.517	0.599	0.671	0.826		0.631	0.730	0.816	1.000	
L.S.D. at 5 %	A: 0.062		B: 0.054		AB: 0.108	A: 0.078		B: 0.051		AB: 0.102
Visnagin %										
Control	0.124	0.161	0.141	0.148	0.144	0.130	0.154	0.166	0.186	0.159
E.M.	0.147	0.176	0.169	0.186	0.170	0.148	0.180	0.189	0.203	0.180
S.A. at 150 ppm	0.170	0.177	0.207	0.217	0.193	0.163	0.172	0.210	0.223	0.192
vit. E at 20 ppm	0.240	0.244	0.283	0.308	0.269	0.280	0.282	0.330	0.376	0.317
E.M. + S.A.	0.192	0.196	0.231	0.307	0.232	0.197	0.215	0.247	0.328	0.247
E.M. + Vit. E	0.265	0.294	0.325	0.339	0.306	0.262	0.295	0.331	0.349	0.309
S.A. + Vit. E	0.299	0.320	0.407	0.430	0.364	0.302	0.327	0.456	0.469	0.389
E.M. + S.A. + Vit. E	0.351	0.439	0.532	0.552	0.469	0.316	0.467	0.515	0.708	0.502
Mean (A)	0.224	0.251	0.287	0.307		0.225	0.262	0.306	0.355	
L.S.D. at 5 %	A: 0.020		B: 0.024		AB: 0.048	A: 0.035		B: 0.020		AB: 0.040

Table (4): Effect of compost, E.M. and/or some stimulant substances treatments (S.A. and vit. E) and their four mixed treatments on N., P. and K. % of *A. visnaga*, during 2011/2012 and 2012/2013 seasons.

Treatment	Compost levels (ton/fed.) (A)									
	1st season					2nd season				
	0	5	7.5	10	Mean (B)	0	5	7.5	10	Mean (B)
N. %										
Control	1.812	2.119	2.248	2.325	2.126	1.801	2.105	2.236	2.320	2.116
E.M.	2.113	2.230	2.368	2.490	2.300	2.085	2.407	2.563	2.655	2.428
S.A. at 150 ppm	1.981	2.148	2.265	2.349	2.186	2.001	2.311	2.442	2.533	2.322
vit. E at 20 ppm	2.031	2.155	2.278	2.390	2.214	2.053	2.364	2.506	2.581	2.376
E.M. + S.A.	2.150	2.271	2.416	2.544	2.345	2.099	2.428	2.593	2.689	2.452
E.M. + Vit. E	2.159	2.293	2.418	2.558	2.357	2.110	2.433	2.615	2.700	2.465
S.A. + Vit. E	2.065	2.185	2.316	2.441	2.252	2.073	2.390	2.541	2.628	2.408
E.M. + S.A. + Vit. E	2.165	2.339	2.429	2.579	2.378	2.130	2.451	2.633	2.720	2.484
Mean (A)	2.060	2.218	2.342	2.460		2.044	2.361	2.516	2.603	
L.S.D. at 5 %	A :0.112		B: 0.033	AB: 0.066		A: 0.085		B: 0.032	AB: 0.064	
P. %										
Control	0.262	0.278	0.293	0.320	0.288	0.274	0.291	0.308	0.329	0.301
E.M.	0.335	0.347	0.356	0.398	0.359	0.339	0.360	0.368	0.399	0.367
S.A. at 150 ppm	0.280	0.295	0.305	0.355	0.309	0.291	0.316	0.328	0.368	0.326
vit. E at 20 ppm	0.301	0.316	0.328	0.379	0.331	0.311	0.330	0.340	0.388	0.342
E.M. + S.A.	0.341	0.355	0.361	0.401	0.365	0.347	0.369	0.377	0.409	0.376
E.M. + Vit. E	0.349	0.369	0.378	0.415	0.378	0.359	0.378	0.390	0.426	0.388
S.A. + Vit. E	0.329	0.339	0.351	0.395	0.354	0.331	0.351	0.362	0.397	0.360
E.M. + S.A. + Vit. E	0.360	0.378	0.390	0.426	0.389	0.372	0.391	0.401	0.431	0.399
Mean (A)	0.320	0.335	0.345	0.386		0.328	0.348	0.359	0.393	
L.S.D. at 5 %	A: 0.014		B: 0.011	AB: 0.022		A: 0.018		B: 0.011	AB: 0.022	
K %										
Control	1.32	1.59	1.85	1.96	1.68	1.42	1.44	1.46	1.69	1.50
E.M.	1.45	1.71	2.00	2.09	1.81	1.63	1.75	2.05	2.11	1.89
S.A. at 150 ppm	1.36	1.61	1.94	2.05	1.74	1.48	1.49	1.56	1.72	1.56
vit. E at 20 ppm	1.40	1.65	1.98	2.05	1.77	1.59	1.69	1.99	2.09	1.84
E.M. + S.A.	1.50	1.72	2.05	2.12	1.85	1.65	1.77	2.08	2.16	1.92
E.M. + Vit. E	1.55	1.78	2.12	2.24	1.92	1.70	1.86	2.15	2.25	1.99
S.A. + Vit. E	1.55	1.75	2.05	2.19	1.89	1.67	1.85	2.10	2.20	1.96
E.M. + S.A. + Vit. E	1.60	1.83	2.17	2.29	1.97	1.76	1.92	2.21	2.31	2.05
Mean (A)	1.47	1.71	2.02	2.12		1.61	1.72	1.94	2.07	
L.S.D. at 5 %	A: 0.09		B: 0.06	AB: N.S.		A: 0.08		B: 0.06	AB: N.S.	

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الملخـص العربي

تأثير بعض المعاملات الزراعية النظيفة على محصول الثمار والمواد الفعالة والتركيب الكيماوي لنبات الخلة البلدي

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أجري هذا البحث في مزرعة كلية الزراعة جامعة المنيا خلال موسمي 2012/2011 و 2013/2012 ويهدف إلى دراسة تأثير سماد الكمبوست (صفر – 5 – 7.5- 10 طن/فدان) وثماني معاملات (الميكروبات الدقيقة النشطة، حمض السالساليك، فيتامين هـ ، الميكروبات الدقيقة النشطة + فيتامين هـ ، الميكروبات الدقيقة النشطة + حمض السالساليك ، حمض السالساليك + فيتامين هـ ، الميكروبات الدقيقة النشطة + حمض السالساليك + فيتامين هـ والكنترول) على محصول الثمار والمواد الفعالة لنبات الخلة البلدي.

أوضحت النتائج أن استعمال الكمبوست (10 طن/فدان) زاد معنوياً محصول الثمار للنبات ومحتوى الثمار من الخلين والفيزناجين والتركيب الكيماوي (النسبة المئوية لعناصر النتروجين والفسفور والبوتاسيوم) مقارنة بالمعاملات الأخرى.

معاملة النباتات بأي من الميكروبات الدقيقة النشطة ،حمض السالساليك ، فيتامين هـ وخليط معاملاتهم أدى إلى زيادة معنوية في محصول الثمار للنبات والمواد الفعالة والنسب المئوية للنتروجين والفسفور والبوتاسيوم مقارنة بمعاملة الكنترول. وكانت أفضل المعاملات في هذا الشأن هي الميكروبات الدقيقة النشطة + حمض السالساليك + فيتامين هـ تليها الميكروبات الدقيقة النشطة + فيتامين هـ.

يمكن التوصية باستخدام 10 طن كمبوست للفدان ومعاملة النباتات بخليط الثلاثة (الميكروبات الدقيقة النشطة + حمض السالساليك + فيتامين هـ) أو خليط الاثنين (الميكروبات الدقيقة النشطة + فيتامين هـ) للحصول على أعلى القيم لمحصول الثمار والمواد الفعالة (الخلين والفيزناجين) لنباتات الخلة البلدي.