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EFFECT OF ORGANIC FERTILIZATION AND PLANT SPACING ON THE GROWTH, OIL PRODUCTION AND ARTEMISININ CONTENT OF SWEET ANNIE (*Artemisia annua*, L.)

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ABSTRACT

This study was carried out during the two successive seasons of 2008 and 2009, to investigate the response of Sweet Annie (*Artemisia annua*) to plant spacing (60 x 40, 60 x 60 or 60 x 80 cm) and fertilization using poultry manure (PM) at the rates of 10, 15 or 20 m^3/fed./season, or cattle manure (CM) at the rates of 15, 25 or 35 m^3/fed./season. The results showed that the widest spacing (60 x 80 cm) increased the mean fresh and dry herb yields/plant, as well as the oil percentage, oil yield/plant and artemisinin content in the leaves. The narrowest spacing (60 x 40cm) produced the highest fresh and dry herb yields/fed., and essential oil yield/fed. The application of PM at the rate of 20 m^3/fed. had the best effect on the mean fresh and dry herb yields (per plant and per fed.), as well as the oil percentage, oil yield per plant and artemisinin content in leaves. The combined treatment of (60 x 80 cm) with PM fertilization at 15 m^3/fed., proved to be the best treatment combined for increasing the fresh and dry herb yields per plant, as well as the oil percentage, the oil yield/plant, and the content of artemisia keton (the main oil component). On the other hand, the highest fresh and dry herb yields/fed., as well as the highest oil yield/fed., were obtained from plants grown at the narrowest spacing (60 x 40 cm), and fertilized with the highest PM rate (20 m^3/fed.).

***Key words*: *Artemisia annua, artemisinin, fertilization, sweet annie*.**

1. INTRODUCTION

Medicinal and aromatic plants are important economic products, which are considered amongst the most important agricultural export products, and they represent significant sources of economic revenue and foreign currency. Egyptian environmental conditions are world-famous for being suitable for growing high quality medicinal and aromatic plants. Recently, there has been an increasing interest in the cultivation and production of medicinal plants in order to cover the growing demand by both local industries and international markets. The Asteraceae (Compositae) is one of the largest and most highly evolved plant families, with a world-wide distribution of about 2500 genera and 25000 species. The genus Artemisia includes about 200 species and some have been cultivated as commercial crops with a wide diversity of uses. Some better known examples include species with antimalarial activity (*A. annua* - annual or sweet wormwood, Sweet Annie), while others are used as culinary spices (*A. dracunculus* - French tarragon), garden ornamentals (*A. abrotanum* - southernwood), insect repellents (*A. vulgaris* - mugwort), or for liquor flavouring (*A. absinthium* - absinthe). However, this study will concentrate on the cultivation of *A. annua* because of its contemporary importance as a source of new and effective antimalarial drugs. Malaria kills a child every few seconds: most of the people who die from malaria are children not yet 5 years of age. Thus, control of this disease has now attained utmost attention, and if such a disease has an herbal solution, then it is surely a boon to mankind. Drug-resistant malaria is a major worldwide public health problem. In Southeast Asia, *Plasmodium falciparum* strains have become resistant to all of the classical antimalarial drugs. Fortunately, these strains are still susceptible to the artemisinin derivatives; derivatives such as artemether and artesunate are now widely used in this region. Artemisinin is a naturally occurring endoperoxide with anti-malarial properties, which has been used clinically as an anti-malaria drug. The range of artemisinin content of *A. annua* harvested from different production areas is wide. The highest content of artemisinin that can be reached in the leaves of *A. annua* is up to 1-2% (on a dry weight basis). Although the content of artemisinin is affected by numerous factors such as geographical conditions, harvesting time, temperature and fertilizer application. Harvesting at the appropriate time is critically important to ensure optimum content of artemisinin in *A. annua*, Zhong Guoyue (1998).

Medicinal and aromatic plants which are grown using organic and biofertilizers are more acceptable than those grown using chemical fertilizers, and are considered to be of higher quality. The demand for healthy organically produced crops (including medicinal plants) is increasingly spreading not only in Egypt but also all over the world. Fertilization using organic and biofertilizers not only provides plants with their nutritional requirements without having an undesirable impact on the environment, but also helps to increase and stabilize soil fertility, and improves the chemical and physical characteristics of the soil.

Plant spacing is an important agronomic factor which affects plant growth and productivity through light interception during photosynthesis. Also, plant spacing has a considerable impact on the exploitation of the photosphere and rhizosphere by the plants, especially when spacing is inadequate, and the plants suffer clustering together. Good plant spacing gives the right plant density, which is the number of plants allowed on a given area of land for optimum yield (Obi, 1991).

2. MATERIALS AND METHODS

This study was conducted at the farm of the Medicinal and Aromatic Plants Research Department (located in El-Kanater El-Khaireya, Kalyoubeya Governorate), Horticulture Research Institute, Agricultural Research Center, Dokki, Giza, during the two successive seasons of 2008 and 2009. The aim of the study was to investigate the effect of some organic fertilizers (poultry and cattle manures) and plant spacing on the growth, oil yield, and artemisinin content of Sweet Annie (*Artemisia annua* L.).

Artemisia annua seeds were sown on the 20^{th} of February in a peat moss medium in the nursery beds, where they germinated within 10-15 days. Two months after sowing the seeds (on the 20^{th} of April, 2008 and 2009 in the two seasons, respectively), when the seedlings were 15-20 cm in height, were transplanted to plots in the open field. The area of each plot was 2.5×3 m containing three rows 60 cm apart and 2.75 meters in length. The chemical analysis of the soil of the experimental field showed that it contained 26.2 ppm N, 106 ppm P_2O_5, 178 ppm K_2O, 5.22 ppm Zn, 2.77 ppm Fe, 2.38 ppm B, 0.66 ppm Mn, and 0.43 ppm Cu.

The seedlings were planted on the rows at three different spacings, *viz*. 40, 60 or 80 cm. Plants grown at each of the three spacings were fertilized using different poultry manure (PM) and cattle manure (CM) application rates. Poultry manure was applied at the rates of 10, 15 and 20 m^3/fed.,

Table (1): Physical and chemical characteristics of the organic manures used for fertilization of Sweet Annie (*Artemisia annua* L.) plants during the 2008 and 2009 seasons.

Organic manure characteristics	Poultry manure (PM)		Cattle manure (CM)	
	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season
Weight of 1 m^3 (kg)	691	546	478	468
Moisture content (%)	7.30	9.33	10.03	9.00
Organic matter (%)	64.21	82.17	59.87	60.24
Organic carbon (%)	39.32	51.30	30.79	36.27
Total N (%)	2.28	3.50	1.78	1.73
C:N ratio	15.2 : 1	13.0 : 1	17.2 : 1	17.7 : 1
NH_3 - N (ppm)	2928.8	3148.9	44.3	64.6
NO_3 – N (ppm)	170.8	214.6	170.4	208.8
Total P (%)	1.05	0.70	0.30	0.64
Total K (%)	0.74	1.78	1.03	1.13
Fe (ppm)	1.689	1.624	1.789	1.590
Mn (ppm)	120.3	158.3	180.5	189.9
Zn (ppm)	81.50	81.90	91.42	97.9
Cu (ppm)	33.7	50.4	39.4	43.4

while CM was applied at rates of 15, 25 and 35 m^3/fed. The poultry and the cattle manures were obtained from the Animal Production Department, El-Kanater El-Khaireya, Agricultural Research Center. The physical and chemical characteristics of the two types of manure are presented in Table (1). The organic fertilization treatments were applied by incorporating the different PM and CM rates into the soil two weeks before transplanting the seedlings (on 5 April 2008 and 2009 in the two seasons, respectively).

This experiment was designed using a factorial split plot design, with 21 treatment combinations (3 plant spacing treatments x 7 organic fertilization treatments, including the control). The organic fertilization treatments were assigned as the main plots in a randomized complete block design with three blocks (replicates), while the plant spacing treatments were assigned as the sub-plots.

All plants received common agricultural practices, including regular watering and manual removal of weeds. At the end of each growing season, the plants were harvested at early bloom stage (on the 20^{th} of October 2008 and 2009 in the two seasons, respectively) by cutting the vegetative parts 10-15 cm above the soil surface.

In each season, data were recorded on herb fresh weight/plant (g), while herb dry weight/plant (g) was recorded after air-drying in a sunny area. The fresh and dry herb yields/fed. (ton) were calculated by multiplying the herb fresh and dry weights/plant, respectively, by the number of plants/fed. Also, the oil percentage in the dry herb was determined using the method described by the British Pharmacopoeia (1963) by hydro distillation of 100 g of dry herb for 4.0 hours. The essential oil yield per plant was calculated in proportion to the herb dry weight/plant. The main constituents of Sweet Annie essential oil were determined by subjecting oil samples (taken from the oil obtained in the first season) to gas liquid chromatographic (GLC) analysis, as recommended by Simon and Quinn (1988). Also, dry leaf samples were chemically analyzed to determine their artemisinin content using the method outlined by Peng *et al*. (2006), by which 95-98% of the artemsinin in the leaves can be extracted.

An analysis of variance (ANOVA) was conducted on the data recorded on fresh and dry herb yields per plant and per fed. The means were compared using the "Least Significant Difference (L.S.D)" test at the 5% level, as described by Little and Hills (1978).

3. RESULTS AND DISCUSSION

3.1.Vegetative growth

3.1.1. Herb fresh and dry weights/plant

The results presented in Tables (2) and (3) show that in both seasons, the highest herb fresh and dry weights per plant were obtained with the widest spacing (60 x 80 cm). The increase in the fresh and dry weight / plant as a result of wide spacing may be attributed to the low density of the plants, allowing the formation of a strong root system, and resulting in a greater uptake of soil, water and nutrients. Also, the wide spacing between the shoots allowed the leaves to capture enough sunlight and air, thus enhancing photosynthesis and metabolic activity, which are subsequently converted into more vegetative growth, resulting in high fresh weights. These results are in harmony with those reported by El-Shaer (1989) on *Foeniculum vulgare* and El-Gendy *et al*. (2001 a) on *Ocimum basilicum*.

The data in Tables (2 and 3) also show that organic fertilization using poultry manure (PM) and cattle manure (CM) had a significant effect on the fresh and dry weights/plant. In both seasons, it is clear that the application of the different organic fertilizer treatments led to significant increases in the herb fresh and dry weights compared to the control. Poultry manure (PM) was clearly more effective than CM in this regard. Also, the recorded values showed steady significant increases as the fertilization rate was raised. Accordingly, the highest herb fresh and dry weights were those of plants fertilized using the highest PM rate (20 m^3/ fed.). The superior effect of PM on fresh and dry weight / plant may be attributed to the higher N content of PM, compared to that of CM, as shown by the results of the chemical analysis of the two organic fertilizers (Table 1). The advantages of using PM (compared to other organic fertilizers) were also detected by El-Ghadban (1998) on *Mentha viridis* and Hassan (2008) on *Thymus vulgaris*.

Regarding the interaction between the effects of plant spacing and organic fertilization on herb fresh and dry weights / plant, the data presented in Tables (1&2) show that significant differences were recorded between the fresh and dry weights of plants receiving the various combinations of these two factors. In both seasons, the heaviest herb fresh and dry weights/plants were those of plants grown at the widest spacing (80 cm) and supplied with PM at the rate of 15 m^3 / fed.

3.2. Fresh and dry herb yields/fed.

The results recorded in the two seasons (Tables 4 and 5) show that the highest mean fresh and dry

Table (2): Effect of spacing and organic fertilization treatments on herb fresh weight/plant (g) of *Artemisia annua* in 2008 and 2009 seasons.

Plant spacing (S)	Herb fresh weight / plant (g)							Means (S)
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	560.67	1325.00	1658.33	1833.33	933.33	1080.00	1333.33	1246.29
60 x 60 cm	433.33	780.00	1216.67	1681.67	648.33	846.67	1020.00	946.67
60 x 80 cm	946.67	1856.67	2571.67	2150.00	1173.33	1488.33	1628.33	1687.86
Means (F)	646.89	1320.56	1815.56	1888.33	918.33	1138.33	1327.22	---
LSD (0.05)								
S	48.10							
F	44.56							
S X F	40.43							
	Second season (2009)							
60 x 40 cm	558.33	1325.00	1663.33	1845.33	930.00	1080.00	1336.67	1248.38
60 x 60 cm	451.67	770.00	1227.67	1653.33	660.00	873.33	1033.33	952.76
60 x 80 cm	948.33	1843.33	2576.67	2260.00	1180.00	1491.67	1650.00	1707.14
Means (F)	652.77	1312.78	1822.56	1919.56	923.33	1148.33	1340.00	---
LSD (0.05)								
S	41.60							
F	35.00							
S X F	24.07							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

Table (3): Effect of spacing and organic fertilization treatments on dry weight / plant (g) of *Artemisia annua* in 2008 and 2009 seasons.

Plant spacing (S)	Dry weight / plant (g)							Means (S)
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	352.33	968.97	1093.00	1156.73	592.20	671.97	852.27	812.49
60 x 60 cm	308.11	559.53	862.33	1231.63	454.77	639.23	717.83	681.92
60 x 80 cm	535.36	1092.87	1560.33	1307.93	706.33	912.40	1025.27	1020.07
Means (F)	398.60	873.79	1171.89	1232.10	584.43	741.20	865.12	---
LSD (0.05)								
S	35.72							
F	31.29							
S X F	29.95							
	Second season (2009)							
60 x 40 cm	343.33	967.67	1093.00	1161.67	588.17	673.00	851.27	811.16
60 x 60 cm	314.33	560.00	863.67	1212.00	466.67	655.47	713.10	683.60
60 x 80 cm	540.00	1096.67	1564.67	1310.33	723.90	899.27	1028.40	1023.32
Means (F)	399.22	874.78	1173.78	1228.00	592.91	742.58	864.25	---
LSD (0.05)								
S	22.66							
F	34.34							
S X F	20.33							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

Table (4): Effect of spacing and organic fertilization treatments on the fresh herb yield/feddan (ton) of *Artemisia annua* in 2008 and 2009 seasons.

Plant spacing (S)	Fresh herb yield / feddan (ton)							Means (S)
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	9.34	22.08	27.63	30.55	15.55	17.99	22.22	2.77
60 x 60 cm	4.81	8.66	13.51	18.65	7.20	9.40	11.33	10.51
60 x 80 cm	7.88	15.47	21.42	13.77	9.77	12.40	13.56	13.47
Means (F)	7.34	15.40	2.086	21.00	10.84	13.26	15.70	---
LSD (0.05)								
S	3.56							
F	4.40							
S X F	5.18							
	Second season (2009)							
60 x 40 cm	9.30	22.08	27.72	30.75	15.49	17.99	22.27	20.80
60 x 60 cm	5.01	8.95	13.95	18.87	7.63	9.90	11.98	10.90
60 x 80 cm	7.90	15.16	21.27	18.63	9.63	12.23	13.54	14.05
Means (F)	7.40	15.39	20.98	22.75	10.92	13.37	15.93	---
LSD (0.05)								
S	3.21							
F	4.36							
S X F	4.96							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

Table (5): Effect of spacing and organic fertilization treatments on the dry herb yield / feddan (ton) of *Artemisia annua* in 2008 and 2009 seasons.

Plant spacing (S)	Dry herb yield / feddan (ton)							Means (S)
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	5.87	16.15	18.21	19.28	9.87	11.20	14.20	13.43
60 x 60 cm	3.42	6.22	9.58	13.68	5.05	7.10	7.975	7.58
60 x 80 cm	4.46	9.11	13.00	10.90	5.89	7.60	8.54	8.50
Means (F)	4.58	10.49	13.59	14.62	6.93	8.63	10.24	---
LSD (0.05)								
S	2.14							
F	3.10							
S X F	4.12							
	Second season (2009)							
60 x 40 cm	5.72	16.13	18.21	19.38	9.80	11.22	14.19	13.52
60 x 60 cm	3.49	6.22	9.60	13.47	5.19	7.28	7.92	7.59
60 x 80 cm	4.50	9.14	13.03	10.91	6.03	7.49	8.57	8.52
Means (F)	4.57	10.49	13.61	14.58	7.00	8.66	10.22	---
LSD (0.05)								
S	2.17							
F	2.00							
S X F	1.98							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

herb yields/fed were produced as a result of growing the plants at the narrowest spacing (60 x 40 cm). This was due to the high plant density at this spacing. These conclusions are in agreement with the findings of Badi *et al.* (2004) on *Thymus vulgaris*.

It can also be seen from the data presented in Tables (4) and (5) that the two highest PM application rates (15 or 20 m^3/fed.) were the most effective fertilization treatments, giving the highest mean fresh and dry herb yields/fed. (in both seasons), with no significant difference between the values obtained with these two application rates. The advantages of using PM (compared to other organic fertilizers) were also detected by Mohsen (2002) on *Ocimum basilicum* cv. Grand Vert, and Vieira *et al*. (2002) on *Mentha villosa* and *Mentha longifolia*.

Regarding the interaction between plant spacing and organic fertilization, it can be seen that in both seasons, the best treatment combination (*i.e.*, giving the highest fresh and dry herb yields/fed.) was using a plant spacing of 60 x 40 cm, with organic fertilization PM at 20 m^3.

3.2. Essential oil production

3.2.1.Essential oil percentage

The synthesis and accumulation of the essential oil in Sweet Annie plants were clearly enhanced by the wide spacing (Table 6). In both seasons, plants grown at the widest spacing (60 x 80 cm) had a significantly higher mean oil percentage, compared to plants grown at the narrowest (60 x 40 cm) and medium spacing (60 x 60 cm). Similar increases in oil percentage as a result of wide plant spacing have been reported by Ahmed (1986) on *Achillea millefolium*, Refai *et al*. (1991) on *Ruta graveolens*, El-Tantawy *et al*. (1992) on *Foeniculum vulgare* Mill., and Morteza *et al.* (2010) on *Valeriana officinalis* L.

The results recorded in the two seasons (Table 6) also show that PM was generally more effective than CM in increasing the essential oil percentage, especially when it was applied at the high rates (15 or 20 m^3/fed.). The increase in the essential oil content of plants fertilized with PM, compared to the control and plants fertilized with CM, is in agreement with the results obtained by Jacoub (1999) and Mohsen (2002) on *Ocimum basilicum*, Salim (2006) on *Salvia officinalis*, and Hassan (2008) on *Thymus vulgaris*.

It is also evident from the results in Table (6) that the interaction between the effects of plant spacing and organic fertilization (using PM and CM) on the oil content in the dry herb was significant. In both seasons, the plants grown at the widest spacing (60 x 80 cm) and fertilized with PM at 20 m^3/fed. had the highest oil percentage.

3.2.2. Essential oil yield/plant

As previously mentioned, planting at the widest spacing (60 x 80 cm) increased the herb fresh and dry weights/plant, as well as the synthesis and accumulation of the essential oil (*i.e.* the oil percentage). Consequently, the results presented in Table (7) show that in both seasons, plants grown at a spacing of 60 x 80 cm gave a higher essential oil yield/plant, compared to the plants grown at 60 x 60 cm or 60 x 40 cm. Similar increases in essential oil yield/plant as a result of wide plant spacing have been reported by Ahmed (1986) on *Achillea millefolium* and Ahmed (1997) on *Nigella sativa*.

The results recorded in the two seasons (Table 7) also show that PM fertilization at the rates of 15 or 20 m^3/fed. increased the oil yield/plant, compared to the values obtained from the control plants, or from plants receiving CM fertilization. These results are in agreement with the findings of Jacoub (1999) and Mohsen (2002) on *Ocimum basilicum* L. cv."Grand Vert", and Hassan (2008) on *Thymus vulgaris*.

Regarding the interaction between the effects of plant spacing and organic fertilization on the oil yield/plant, the data in Table (7) show that the highest oil yield/plant was obtained from plants grown at the widest spacing (60x70 cm) and supplied with the medium PM rate (15 m^3/fed).

3.3. Essential oil yield/fed.

The essential oil yield/fed. was evidently more influenced by plant density (*i.e.*, the number of plants/fed.) than by the essential oil yield/plant, or the herb weight/plant. Accordingly, Table (8) shows that growing the plants at the narrowest spacing (60 x 40 cm), gave the highest number of plants/fed., also resulted in a significantly higher essential oil yield / fed., compared to values recorded with wider plant spacing (60 x 60 cm, or 60 x 80 cm). Increases in the essential oil yield / fed. as a result of narrow plant spacing were also reported by Ahmed (1986) on *Achillea millefolium*, Kalyan *et al*. (1989) on *Mentha arvensis*, Shalaby and Razin (1992) on *Thymus vulgaris*, Shalaby *et al*. (1993) on *Melissa officinalis*, Ram *et al*. (1998) on *Tagetes minuta* and El-Gendy *et al.* (2001b) on *Ocimum basilicum* var. Grand Vert.

The results recorded in the two seasons (Table 8) also show that the different organic fertilization treatments increased the oil yield/fed. significantly, compared to the control. Fertilization using PM was most effective in

Table (6): Effect of spacing and organic fertilization treatments on the essential oil content (%) in the dry herb of *Artemisia annua* in 2008 and 2009 seasons.

Plant spacing (S)	Essential oil content (%)							Means
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	0.75	0.88	0.92	0.94	0.82	0.86	0.91	0.86
60 x 60 cm	0.73	0.80	0.86	0.95	0.78	0.81	0.83	0.82
60 x 80 cm	0.81	0.92	1.09	1.02	0.86	0.92	0.94	0.94
Means (F)	0.76	0.86	0.96	0.97	0.82	0.86	0.89	---
LSD (0.05)								
S	0.06							
F	0.04							
S X F	0.05							
	Second season (2009)							
60 x 40 cm	0.78	0.89	0.94	1.01	0.85	0.91	0.92	0.89
60 x 60 cm	0.76	0.81	0.88	0.97	0.82	0.84	0.86	0.85
60 x 80 cm	0.81	0.91	1.16	1.06	0.88	0.93	0.94	0.95
Means (F)	0.77	0.87	0.99	1.01	0.85	0.89	0.91	---
LSD (0.05)								
S	0.04							
F	0.06							
S X F	0.03							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

Table (7): Effect of spacing and organic fertilization treatments on the essential oil yield/ plant (ml) of *Artemisia annua* in 2008 and 2009 seasons.

Plant pacing (S)	Essential oil yield / plant (ml)							Means (S)
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	2.64	8.52	10.05	10.87	4.85	5.77	7.75	7.21
60 x 60 cm	2.24	4.47	7.41	11.70	3.54	5.17	5.95	5.78
60 x 80 cm	4.33	10.05	17.00	13.34	6.07	8.39	9.63	9.83
Means (F)	3.07	7.68	11.48	11.97	4.82	6.44	7.78	---
LSD (0.05)								
S	0.84							
F	0.68							
S X F	0.53							
	Second season (2009)							
60 x 40 cm	2.61	8.61	10.27	11.73	5.00	6.12	7.83	7.45
60 x 60 cm	2.39	4.54	7.60	11.76	3.83	5.51	6.13	5.96
60 x 80 cm	4.37	9.98	18.15	13.89	6.37	8.36	9.67	10.11
Means (F)	3.12	7.71	12.01	12.46	5.07	6.66	7.88	---
LSD (0.05)								
S	2.50							
F	1.97							
S X F	3.19							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively

Table (8): Effect of spacing and organic fertilization on the essential oil yield/fed. (kg) of *Artemisia annua* in 2008 and 2009 seasons.

Plant spacing (S)	The essential oil yield / fed. (L)							Means (S)
	Fertilization treatments (F)*							
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
	First season (2008)							
60 x 40 cm	43.45	142.10	167.58	181.21	76.98	91.83	120.73	117.70
60 x 60 cm	24.64	49.73	82.39	130.00	38.40	58.95	64.60	64.10
60 x 80 cm	35.24	81.96	132.62	91.05	49.44	66.14	76.03	76.07
Means (F)	34.44	91.26	127.53	134.09	54.94	72.30	87.12	---
LSD (0.05)								
S	13.66							
F	15.51							
S X F	11.65							
	Second season (2009)							
60 x 40 cm	44.63	144.06	171.22	196.18	83.64	102.44	131.46	124.81
60 x 60 cm	26.54	50.60	84.44	129.27	41.13	61.17	68.13	65.90
60 x 80 cm	36.59	83.46	137.24	100.46	53.08	69.94	81.12	80.27
Means (F)	35.92	92.71	130.97	141.97	59.28	77.85	93.57	---
LSD (0.05)								
S	15.02							
F	16.64							
S X F	12.22							

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

Table (9): Effect of spacing and organic fertilization on the components (%) of the essential oil of *Artemisia annua* L. in 2008 season.

Plant spacing (S)	The components (%) of the essential oil Fertilization treatments (F)*							Means (S)
	Control	**PM1**	**PM2**	**PM3**	**CM1**	**CM2**	**CM3**	
				α - Thujene				
60 x 40 cm	0.683	0.653	0.500	0.562	0.778	0.767	1.131	**0.724**
60 x 60 cm	1.043	1.574	1.310	1.075	1.484	1.024	0.978	**1.212**
60 x 80 cm	2.683	0.834	0.722	0.938	1.810	0.606	0.661	**1.179**
Means (F)	**1.469**	**1.020**	**0.844**	**0.858**	**1.357**	**0.799**	**0.923**	**1.038**
				α - Pinene				
60 x 40 cm	1.079	0.821	1.087	2.034	1.279	1.607	2.421	**1.475**
60 x 60 cm	2.306	2.341	3.101	1.971	1.824	1.486	2.075	**2.157**
60 x 80 cm	2.893	1.360	1.142	1.089	2.014	1.557	1.697	**1.678**
Means (F)	**2.092**	**1.507**	**1.776**	**1.698**	**1.705**	**1.550**	**2.064**	**1.770**
				Camphene				
60 x 40 cm	4.242	2.336	2.045	1.275	3.540	1.865	3.229	**2.647**
60 x 60 cm	4.731	6.176	1.594	4.155	3.555	2.145	5.969	**4.046**
60 x 80 cm	3.461	1.536	2.893	3.870	2.556	1.475	4.380	**2.881**
Means (F)	**4.144**	**3.349**	**2.177**	**3.100**	**3.217**	**1.828**	**4.526**	**3.191**
				β - pinene				
60 x 40 cm	4.713	2.575	5.737	1.986	1.934	4.501	1.939	**3.340**
60 x 60 cm	1.807	8.149	3.049	2.616	7.917	4.760	4.915	**4.744**
60 x 80 cm	3.688	3.004	2.524	5.564	2.660	6.579	2.177	**3.742**
Means (F)	**3.402**	**4.576**	**3.770**	**3.388**	**4.170**	**5.280**	**3.010**	**3.942**
				α-Terpinene				
60 x 40 cm	2.253	2.618	3.820	2.089	3.872	1.984	1.707	**2.620**
60 x 60 cm	2.827	1.910	1.451	1.719	0.903	1.457	1.735	**1.714**
60 x 80 cm	2.381	1.179	1.348	1.569	3.095	1.248	0.869	**1.669**
Means (F)	**2.487**	**1.902**	**2.206**	**1.792**	**2.623**	**1.563**	**1.437**	**2.001**
				1,8 Cineol				
60 x 40 cm	16.524	7.692	9.858	9.052	14.422	11.941	8.938	**11.203**
60 x 60 cm	10.483	14.060	10.378	11.984	12.035	14.447	8.025	**11.630**
60 x 80 cm	10.747	14.085	9.842	10.955	14.109	10.394	9.654	**11.398**
Means (F)	**12.584**	**11.945**	**10.026**	**10.663**	**13.522**	**12.260**	**8.872**	**11.410**
	Artemisia keton							
60 x 40 cm	21.525	35.764	36.319	40.591	30.440	33.069	36.889	**33.513**
60 x 60 cm	19.723	24.847	30.788	39.036	21.121	30.833	36.804	**29.021**
60 x 80 cm	29.432	38.390	44.113	43.326	33.729	39.556	40.616	**38.451**
Means (F)	**23.560**	**33.000**	**37.073**	**40.984**	**28.430**	**34.486**	**38.103**	**33.662**

Continued

Table 9:Continued

Plant spacing (S)	The components (%) of the essential oil Fertilization treatments (F)*							Means (S)
	Control	PM1	PM2	PM3	CM1	CM2	CM3	
Terpinolene								
60 x 40 cm	4.363	4.034	3.504	2.592	5.368	3.145	3.415	**3.774**
60 x 60 cm	5.211	3.175	3.638	2.866	3.037	3.923	2.528	**3.482**
60 x 80 cm	6.962	2.820	3.458	3.035	3.893	1.939	3.467	**3.653**
Means (F)	**5.512**	**3.343**	**3.533**	**2.831**	**4.099**	**3.002**	**3.136**	**3.636**
Camphor								
60 x 40 cm	13.850	10.840	12.896	10.709	11.650	20.460	23.392	**14.828**
60 x 60 cm	23.192	15.495	18.115	16.472	14.624	22.643	18.273	**18.431**
60 x 80 cm	12.106	12.545	10.118	9.963	13.416	17.046	15.837	**13.004**
Means (F)	**16.450**	**12.960**	**13.709**	**12.381**	**13.230**	**20.049**	**19.167**	**15.421**
Borneol								
60 x 40 cm	2.825	2.294	1.546	5.558	5.097	3.084	2.497	**3.271**
60 x 60 cm	3.161	2.456	7.729	1.522	3.770	1.827	2.122	**3.226**
60 x 80 cm	1.414	2.053	3.185	2.242	3.154	2.119	4.254	**2.631**
Means (F)	**2.466**	**2.2676**	**4.153**	**3.107**	**4.007**	**2.343**	**2.957**	**3.043**
Eugenol								
60 x 40 cm	3.063	2.805	2.022	1.832	2.294	1.531	1.145	**2.098**
60 x 60 cm	2.183	1.767	1.904	1.463	1.638	1.227	1.852	**1.719**
60 x 80 cm	2.090	2.890	2.336	1.822	1.166	1.549	1.499	**1.907**
Means (F)	**2.445**	**2.487**	**2.087**	**1.705**	**1.699**	**1.435**	**1.498**	**1.908**
B Caryophellene								
60 x 40 cm	3.699	4.345	5.600	3.872	6.040	3.278	2.892	**4.246**
60 x 60 cm	4.862	4.826	5.035	4.786	3.600	4.129	3.815	**4.436**
60 x 80 cm	6.232	4.690	3.646	5.015	4.189	3.596	3.989	**4.479**
Means (F)	**4.931**	**4.620**	**4.760**	**4.557**	**4.609**	**3.667**	**3.565**	**4.387**
Unknown Components								
60 x 40 cm	21.181	23.223	15.066	17.848	13.286	12.768	10.405	**16.253**
60 x 60 cm	18.471	13.224	14.908	10.335	24.492	10.099	10.909	**14.634**
60 x 80 cm	15.911	14.614	14.673	10.612	14.209	12.336	10.900	**13.322**
Means (F)	**18.521**	**17.020**	**14.882**	**12.931**	**17.329**	**11.734**	**10.738**	**14.736**

*** PM1, PM2 and PM3 = Poultry manure at 10, 15 and 20 m3 / fed., respectively.**
CM1, CM2 and CM3 = Cattle manure at 15, 25 and 35 m3 / fed., respectively.

Table (10): Effect of spacing and organic fertilization treatments on the rtemisinin content (%) in the dry leaves of *Artemisia annua* in 2008 season.

Plant spacing (S)	Artemisinin content (% of dry leaves) Fertilization treatments (F)*			Means (S)
	Control	PM3	CM3	
60 x 40 cm	1.09	2.26	1.91	1.75
60 x 60 cm	1.18	3.83	1.88	2.29
60 x 80 cm	1.39	3.84	2.31	2.51
Means (F)	1.22	3.31	2.03	---

PM3: poultry manure at 20 m3 / fed. **CM3: cattle manure at 35 m3 / fed.**

increasing the oil yield/fed. when it was applied at the rates of 15 or 20 m^3. The above results are in agreement with the findings of El-Ghadban (1998) on *Mentha viridis* and Mohsen (2002) on *Ocimum basilicum* L. cv."Grand Vert".

It is also evident from the results in Table (8) that the interaction between the effects of plant spacing and organic fertilization on the oil yield/fed. was significant. In both seasons, the plants planted at the narrowest spacing (60 x 40 cm) and fertilized with PM at 20 m^3/fed. gave the highest oil yield/fed.

3.4. Essential oil components

Chromatographic analysis of oil samples extracted from Sweet Annie plants in the first season (Table 9) show that artemisia keton was the most important essential oil component (with contents of 19.723-44.113%), followed by camphor (with contents of 9.963-23.392%), then 1,8 Cineol (with contents of 7.692-16.524%). Also, the data in Table (6) show that plant spacing had a considerable effect on the artemisia keton content. The maximum mean content (38.451%) was obtained with a spacing of 60 X 80 cm, followed by 60 x 40 cm (with a value 33.513%), then 60 x 60 cm (with a value of 29.021%). The data in Table (9) also show that the plants fertilized with PM at 20 m^3/fed. had the highest artemisia keton content (40.984%), followed by the plants receiving PM at 15 m^3/fed. (with an artemisia keton content of 37.073%). Plants grown at a spacing of 60 x 80 cm and fertilized with PM at 15 m^3/fed. had a higher artemisia keton content (44.113%) than plants receiving any other treatment combination.

Artemsinin content in the leaves (%)

It can be seen from the data in Table (10) that the artemisinin content in the leaves of *Artemisia annua* was affected by the plant spacing treatments. The widest spacing (60 x 80 cm) gave the highest mean artemisinin content (2.51%). Moreover, decreasing the plant spacing caused a steady reduction in the artemisinin content. Similar increases in the artemisinin content as a result of wide plant spacing have been reported by Galanopoulou *et al.* (2002) and Mert *et al.* (2002) on *Artemisia annua* L.

The data presented in Table (10) also show that organic fertilization (using CM or PM) caused a marked increase in the artemisinin content in *Artemisia annua* leaves, compared to that of the control plants. The highest mean artemisinin content (3.31%) was obtained in plants fertilized with poultry manure at 20 m^3/fed., whereas plants fertilized with cattle manure at 35 m^3/fed. had an artemisinin content of 2.03%.

Regarding the interaction between the effects of plant spacing and organic fertilization on the artemisinin content, the data presented in Table (10) show that considerable differences were recorded between the artemisinin contents of plants receiving the various combination of these two factors. The highest artemisinin content (3.84%) was obtained in the leaves of plants grown at the widest spacing (60 x 80 cm) and supplied with the highest PM rate (20 m^3/fed.), followed by the plants receiving the same PM rate (20 m^3/fed.), but combined with the medium plant spacing (60 x 60 cm), which gave an artemisinin content of 3.83%.

From the aforementioned results, it can be concluded that the plants grown at the widest spacing (60 x 80 cm) and fertilized with PM at 15 m^3/fed. gave the highest fresh and dry herb yields/plant, as well as the highest essential oil percentage, the highest oil yield/plant, and the highest content of artemisia keton (the main oil component) in the oil. However, growing the plants at the narrowest spacing (60 x 40 cm) considerably increased plant density (the number of plants/fed.). Consequently, plants grown at this spacing (60 x 40 cm) and fertilized with PM at 20 m^3/fed. gave the highest fresh and dry herb yields/fed., as well as the highest essential oil yield/fed.

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تأثير التسميد العضوى ومسافات الزراعة على النمو وإنتاج الزيت ومحتوى الأرتيميسينين فى نبات الأرتيميسا (*Artemisia annua*, **L.**)

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ملخص

أجريت هذه الدراسة فى الموسمين المتتالين 2008 و2009 لدراسة تأثير كل من مسافات الزراعة (60×40، 60×60، 60×80 سم) والتسميد العضوى باستخدام كل من سماد الدواجن بمعدلات 10، 15، 20م^3/فدان وسماد الماشية بمعدلات 15، 25، 35م^3/فدان على نمو وإنتاج الزيت ومحتوى الأرتيميسينين فى نبات الأرتيميسا.

أوضحت النتائج أن مسافة الزراعة الواسعة (60×80 سم) أدت الى زيادة متوسط وزن العشب الطازج والجاف للنبات والنسبة المئوية للزيت ومحصول الزيت للنبات وزيادة محتوى الأرتيميسينين فى أوراق النبات، وكانت المسافة 60×40 سم الأفضل تأثيراً على محصول الزيت والعشب الطازج والجاف للفدان. هذا و كان سماد الدواجن بمعدل 20م^3/فدان هو أفضل معاملات التسميد حيث أدى الى زيادة محصول العشب الجاف والطازج لكل من النبات والفدان، والنسبة المئوية للزيت ومحصول الزيت للنبات ومحتوى الأرتيميسينين فى أوراق النبات. أدى الجمع بين أوسع مسافات زراعة (60 × 80 سم) والتسميد العضوى بسماد دواجن بمعدل 15 م^3 /فدان إلى إعطاء أفضل النتائج من حيث زيادة محصول العشب الطازج والجاف للنبات والنسبة المئوية للزيت ومحصول الزيت للنبات ومحتوى الزيت من الأرتيميسا كيتون (المكون الرئيسى للزيت) . من ناحية أخرى كانت الزراعة على مسافات 60×40 مع إضافة سماد الدواجن بمعدل 20 م^3/فدان هى أفضل المعاملات من حيث الزيادة فى محصول العشب الطازج والجاف للفدان وكذلك محصول الزيت للفدان.

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