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Yield and Chemical Composition of Essential Oil of *Achillea millefolium* L. as Affected by Harvest Time

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Achillea millefolium is an important medicinal and aromatic plant and is grown commercially for production of essential oil in many countries including Egypt. The plant extract and essential oil possess antioxidant, antimicrobial, antispasmodic and antitumor properties. We hypothesized that the essential oil content and composition may be influenced by harvest time and environmental conditions. Thus, flowering heads yield and the essential oil content and quantitative composition were investigated during different harvest times in the Experimental Station of National Research Centre at Nubaria, Beheira Governorate, West of Nile Delta, Egypt under drip irrigation. Flowering heads yield and essential oil composition varied according to harvest time. The highest essential oil yield was observed at the second harvest on10 April and 5 May of the first and second seasons, respectively. The essential oils at the different harvest times were characterized by containing monoterpenes with high amounts of β-pinene (24.1–54.6%) and sabinene (3.1–7.4%). The most abundant sesquiterpenes were chamazulene (10.1–26.7%) followed by geramacrene-D (1.3–10.3%) and β-caryophyllene (0.9–6.4%). Plants harvested on 21 February provided essential oil with relatively high concentration of chamazulene and germacrene-D. Chamazulene and germacrene-D decreased gradually at the later harvests, while β-pinene and limonene reached the maximum concentration in plants harvested on15 May. These results indicate that flowering heads yield and essential oil content and composition can be influenced by harvest time and climatic conditions which can be useful to producers and processors of *Achillea millefolium*.

Keywords: Essential oil, *Achillea millefolium,* Harvest time, Sandy soil.

Introduction

Achillea millefolium L. (common yarrow) belongs to family *Asteraceae* and is widely used in folk medicine because of its numerous pharmacological properties, such as anti-inflammatory [1], antispasmodic [2], antiseptic [3], diaphoretic, anti-swelling, and antihypertensive [4], diuretic and antidiarrheal [5] and emmenagogue [6]. Nowadays, common yarrow is used in healing ointments applied to wounds [7] and in modern medicine for reducing ulcer size [8]. It also has antitumor [9], antioxidant [10], antimicrobial [3], antifungal [11], anti-mutagenic [12], liver protective [13], muscle relaxant [14], gastric anti-secretory and gastroprotective activities [15].

The yarrow flower contains 0.2–0.5% essential oil, while the leaves and stem parts contain only 0.02–0.07% [16]. The essential oil was characterized by containing sesquiterpenes with high amounts of chamazulene, β-caryophellene, and geramacrene–D, while the major monoterpenes were β-pinene, sabinene, and 1,8 cieneole [17], α-pinene and camphor [18], myrcene, limonene and camphene [19] and borneol [20].

The herb yield and essential oil content and composition of some aromatic plants could be influenced by harvest time and ecological and climatic conditions, as demonstrated for thyme, oregano and basil [21, 22]. Knowledge of the influence that harvesting time and environmental

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factors have on essential oil content is insufficient. The general assumption for essential oil plants is that the material is best collected when the plant part in question has reached its optimal state of development; based on this assumption herbs are commonly collected at the flowering stage [23]. The variation in the content of secondary metabolites is important for the interaction of plants with pathogens and herbivores, but also for meeting industry standards for a particular essential oil profile. Several studies reported that the concentration of terpenoids in aromatic plants varies during the day and season [24, 21, 25, 26]. Although monoterpenes predominate in most essential oils, many also contain sesquiterpenes. This study aimed to investigate the herb yield and essential oil content and quality composition of *Achillea millefolium* at four harvest times under desert conditions in newly reclaimed soil in Egypt.

Materials and Methods

The field experiments were carried out at the Agricultural Experimental Station of National Research Centre (Nubaria, Beheira Governorate, West of Nile Delta, Egypt) during two successive seasons of 2007–2008 and 2008–2009 under drip irrigation.

The physical and chemical properties of the soil were determined [27] (Table 1).

TABLE 1. Physical and chemical properties of the soil at the experimental site.

Physical properties							
Season	**Very coarse sand**	**Coarse sand**	**Medium sand**	**Fine sand**	**Very fine sand**	**Silt+Clay**	**Texture**
	---------- % ----------						
2007–2008	34.77	35.31	0.48	15.98	12.45	1.01	Sandy
2008–2009	31.52	39.25	0.43	16.57	11.31	0.92	Sandy

Chemical properties										
Season	**pH (2.5:1)**	**E.C. (1:1) (dSm-1)**	**Cations**				**Anions**			
			----------(meq/l)----------							
			Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**CO_3^{-}**	**HCO_3^{-}**	**Cl^{-}**	**So_4**
2007–2008	7.71	0.2	0.8	0.4	0.8	0.1	-	0.8	0.1	0.3
2008–2009	7.96	0.4	1.5	1.0	1.3	0.2	-	2.1	1.0	0.9

Weather information at the experimental farm area during the growing period is presented in Table 2.

The layout of the field experiment was a randomized complete block design with three replications. Two weeks before planting, the soil was cultivated and divided into plots and drip lines were also installed at this time. The plot size was 22.0 by 7.5 m, with 10 rows spaced 75 cm apart and 50 cm between plants in row (45 plants per row). Compost at 75 ton ha^{-1}, calcium super phosphate (15.5% P_2O_5) at 250 kg P_2O_5 ha^{-1}, potassium sulfate (48.5% K_2O) at 250 kg K_2O ha^{-1}, and agricultural sulphur (99.9%) at 740 kg S ha^{-1}were added prior to soil preparation. Ammonium sulphate at 495 kg N ha^{-1} was divided into four applications and added after planting and after the first, second and third harvest.

Rhizomes of *Achillea millefolium* L. plants were obtained from the Experimental Farm of the Faculty of Pharmacy, Cairo University, Egypt. On 3 October in the first season and 19 October in the second season, divided plants of *Achillea millefolium* were planted, one plant every 50cm adjacent to the drippers in the drip lines. Some plants did not survive; two weeks after planting the empty places were replanted. Plants were watered with a drip irrigation system at 2 L h^{-1} for 6 h a day, 4 days a week.

The flowering heads of *Achillea millefolium* plants were collected at four harvest times. The first harvest was on 21 February and 10 March in the first and the second seasons, respectively. The second harvest was on 10 April and 5 May, the third was on 15 May and 21 July and the fourth was on 7 July and 30 September. Fresh and dry

TABLE 2. Monthly average of metrological data at the experimental area during 2007-2008 and 2008-2009 seasons

Month	2007–2008 season				2008–2009 season			
	Air temperature °C			R.H. %	Air temperature °C			R.H. %
	Max.	Min.	Average		Max	Min	Average	
Sep	30.0 2	22.00	27.02	61.10	30.63	20.47	25.60	67.80
Oct	30.64	20.34	25.48	63.39	25.84	15.71	21.06	71.10
Nov	25.61	15.37	20.50	56.86	23.90	13.27	18.77	70.23
Dec	20.89	11.54	16.21	59.83	19.71	9.68	14.87	71.58
Jan	16.06	7.48	11.90	73.61	18.81	8.03	13.52	70.26
Feb	16.28	6.86	11.72	74.72	19.07	7.82	13.54	67.54
Mar	23.42	10.16	16.94	66.58	19.55	8.65	14.19	64.29
Apr	23.52	13.72	18.62	66.07	23.43	11.67	17.63	68.93
May	26.16	14.97	20.71	63.77	25.45	14.81	20.29	65.55
Jun	30.03	18.47	24.37	67.87	29.90	19.77	24.93	65.43
Jul	29.94	21.10	25.58	70.29	30.29	21.90	26.13	71.06
Aug	–	–	–	–	30.35	20.90	25.68	69.32
Sep	–	–	–	–	29.63	20.23	25.03	67.23

Source: Metrological data of Cairo, Egypt.

weight of flowering heads (g plant^{-1} and ton ha^{-1}) were recorded.

Essential oil yield (ml plant^{-1} and L ha^{-1}) was determined by hydro-distillation for 3 h of 150 g fresh flowering heads, in three replicates using Clevenger-type apparatus, for each harvest time, according to the Egyptian Pharmacopoeia (1984) [28]. The obtained essential oil was dehydrated over anhydrous sodium sulfate, and then analyzed by gas liquid chromatographic (GLC) analysis. The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyl (reqiv.) polysillphenylene- siloxan 30-m by 0.25-mm ID by 0.25-µm film. Temperature program ramp increased by a rate of 80ºC/min from 70 to 200ºC. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min, and air at 330 ml/min. Detector and injector temperatures were 300ºC and 250ºC, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil. The area of each peak was first calculated by an automatic integrator. The areas were then summed. The total area of the peaks represented the whole sample. The percentage of each component was the ratio between its peak areas to the total peak area, multiplied by 100. The identification of individual essential oil compounds was achieved by matching their retention times with those of authentic samples injected under the same conditions.

The data were analyzed using the MSTAT-C program [29]. Least significant difference test was applied at 0.05 probability level to compare mean treatments.

Results and Discussion

Fresh and dry biomass yields

Data presented in Table 3 revealed that harvest time had a significant effect on fresh and dry weights of flowering heads (g plant^{-1} and ton ha^{-1}). In the first season the maximum fresh weight (83.7g plant^{-1} and 2.27 ton ha^{-1}) and dry weight (35.3g plant^{-1} and 0.96 ton ha^{-1}) were recorded at the third harvest on15 May. During the second season, the second harvest in May provided the greatest yield of flowering heads. In both seasons, fresh and dry weight yield gradually increased with plant age to reach their maximum values in May. The average temperatures in the experimental area were 20.7 and 20.3ºC in the first and second seasons, respectively. Generally, these findings agree with those obtained by Aziz (2004) [17], who stated that the greatest fresh and dry weight values of *Achillea millefolium* flowering heads were obtained at the third harvest on15 April. The influence of harvest time on fresh

and dry weight of flowering heads may be a result of available day-length, temperature, and sunlight accumulations [30]. Indeed, previous research has demonstrated significant effects of harvest time on oil yields and composition in other plant species, e.g., dill (*Anethum graveolens*) [31, 32], *Dracocepholum moldavica*) and *Origanum majorana* [33], lemongrass (*Cymbopogon flexuosus*) [34], spearmint ('Scotch' spearmint [*Mentha* × *gracilis* Sole]) and 'Native' spearmint [*M. spicata* L.]) [35].

TABLE 3. Effect of harvest time on flowering head production of *Achillea millefolium* during the 2007–2008 and 2008–2009 seasons.

	2007–2008 season			
	Fresh weight		Dry weight	
Harvesting time	g plant^{-1}	ton ha^{-1}	g plant^{-1}	ton ha^{-1}
1st 21 Feb	40.00	1.09	10.50	0.30
2nd 10 Apr	47.53	1.28	15.10	0.42
3rd 15 May	83.73	2.27	35.33	0.96
4th 7 Jul	79.30	2.15	28.20	0.77
LSD at 5%	5.66	0.05	3.93	0.04
	2008–2009 season			
1st 10 Mar.	40.00	0.44	10.93	0.12
2nd 5 May	114.20	1.26	35.20	0.39
3rd 21 Jul.	58.77	0.65	18.77	0.21
4th 30 Sep.	32.10	0.35	11.10	0.12
LSD at 5%	6.33	0.04	7.55	0.01

Essential oil production

The essential oil yields from the four harvests of both seasons are shown in Table 4. Time of harvest had a significant effect on the essential oil percentage and yield (ml plant^{-1} and L ha^{-1}). In the first season, the highest essential oil percentage was obtained from the third harvest on 15 May, while in the second season the fourth harvest on 30 September had the highest essential oil percentage. Moreover the highest oil yield (ml plant^{-1} and L ha^{-1}) was observed at the third harvest on 15 May in the first season and at the

TABLE 4. Effect of harvest time on essential oil production of Achillea millefolium flowering heads during the 2007–2008 and 2008–2009 seasons.

Harvest time	2007–2008 season		
	%	m plant^{-1}	L ha^{-1}
1st 21 Feb	0.067	0.027	0.741
2nd 10 Apr	0.144	0.068	1.894
3rd 15 May	0.186	0.156	4.308
4th 7 Jul	0.183	0.145	4.014
LSD at 5%	0.001	0.006	0.005
	2008–2009 season		
	%	ml plant^{-1}	L ha^{-1}
1st 10 Mar	0.082	0.033	0.906
2nd 5 May	0.127	0.145	4.011
3rd 21 Jun	0.148	0.087	2.406
4th 30 Sep	0.177	0.057	1.571
LSD at 5%	0.004	0.009	0.005

second harvest on 5 May in the second season.

The maximum essential oil contents depend not only on flower development, but also on temperature, relative humidity, and duration of sunshine [36, 37]. Moreover, the content of essential oil is known to depend considerably upon extrinsic and intrinsic factors, including soil and climatic conditions, plant ontogenesis phases, and harvest [25]. Thus, we can explain the oil content variations from year to year by the variations in weather conditions.

Essential oil composition

The results in Table 5 show that the major components at different harvest times were β-pinene followed by chamazulene, limonene and germacrene-D; this agrees with previous reports on this plant [17, 19].

The essential oil of *Achillea millefolium* at the different harvest times was characterized by containing sesquiterpenes with high amounts of chamazulene (10.1–26.7%), followed by germacrene-D (1.3–10.3%) and β-caryophyllene (0.9–6.4%). The major monoterpenes were β-pinene (24.1–54.6%) and sabinene (3.1–7.4%). Plants harvested on21 Februaryyieldedessential oil with high relative concentrations of chamazulene and germacrene-D. The concentrations of the latter compounds decreased with increasing plant age while β-pinene and limonene were greatest in oil from plants harvested on15 May. Amounts of sabinene and β-caryophellene were greatest at the first harvest on 21 February and decreased gradually with the later harvests. Moreover, amounts of α-terpinene and camphor were low in the first harvest on21February and reached their highest relative concentrations at the third and fourth harvests, respectively.

Silvestre et al. (1997) [38] found that the essential oil composition of Eucalyptus-globulus Labill may vary considerably throughout the year. Marotti et al. (1994) [39] reported that the quantitative composition of the essential oils of peppermint plants is influenced by harvest time and plant age. Also, the cultivation season and day length influence peppermint oil composition [40].

Conclusion

The flowering head production and the essential oil content and composition of *Achillea millefolium* are influenced by harvest time, and ecological and climatic conditions. Thus, in the newly reclaimed lands of Egypt, we recommend harvesting *Achillea millefolium* plants in May for highest essential oil production.

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TABLE 5. Effect of harvest time on essential oil composition of Achillea millefolium flowering heads during the 2008–2009 season.

Oil composition	10 Mar %	R.T.	5 May %	R.T.	21 Jul %	R.T.	30 Sep %	R.T.
α-pinene	1.4	2.48	0.4	2.39	0.70	2.40	0.5	2.41
Sabinene	7.4	2.92	3.1	2.50	4.20	2.51	4.3	2.52
β-pinene	24.1	3.03	53.0	3.08	54.6	3.10	53.0	3.13
Myrcene	0.4	3.40	0.5	3.41	1.0	3.42	0.7	3.44
Limonene	6.4	3.68	8.8	3.71	11.9	3.73	9.8	3.74
1,8 cineole	1.0	3.92	1.1	3.93	1.6	3.94	1.2	3.96
α-terpinen	2.2	5.41	1.3	5.41	3.0	5.43	5.1	5.47
Camphor	1.3	5.83	1.5	5.85	3.7	5.87	2.8	5.88
Bornyl acetate	0.8	6.10	1.3	6.12	2.6	6.14	2.4	6.16
Chamazulene	26.7	9.15	13.5	9.13	10.1	9.12	11.6	9.51
Germacrene-D	10.3	9.57	4.6	9.94	1.3	9.90	2.2	9.94
β-caryophellene	6.4	10.17	2.1	10.15	1.0	10.13	0.9	10.14
Total identified	88.4		91.2		95.7		94.5	

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المحصول و التركيب الكيميائي للزيت الطيار لنبات الآشيليا تحت تأثير ميعاد الحصاد

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يعتبر نبات الآشيليا أحد النباتات الطبية و العطرية الهامة و التي تزرع تجاريا في مصر و العديد من البلدان من أجل إنتاج الزيت الطيار. يعطي مستخلص النبات و كذلك الزيت الطيار نشاط مضاد للأكسدة، الميكروبات، التشنجات و الأورام. بافتراض أن محتوى و مكونات الزيت الطيار يمكن أن تتأثر بميعاد الحصاد و الظروف البيئية المحيطة لذلك تمت دراسة محصول الرؤوس الزهرية و محتوى و مكونات الزيت الطيار خلال مواعيد الحصاد المختلفة تحت ظروف الري بالتنقيط و ذلك محطة التجارب الخاصة بالمركز القومي للبحوث بمدينة النوبارية – محافظة البحيرة- غرب الدلتا – مصر. لوحظ وجود إختلافات في محصول الرؤوس الزهرية و مكونات الزيت الطيار تبعا لميعاد الحصاد. تم تسجيل أعلى محصول من الزيت الطيار في الميعاد الثاني للحصاد (١٠ ابريل و ٥ مايو) في الموسمين الأول و الثاني على التوالي. تميز الزيت الطيار في جميع مواعيد الحصاد بإحتوائه على تربينات أحادية هي β-pinene (٢٤,١-٥٤,٦٪) و sabinene (٣,١-٧,٤٪) بينما السيسكوتربينات الرئيسية كانت chamazulene (١٠,١-٢٦,٧٪) يليه geramacrene-D (١,٣-١٠,٣٪) و β-caryophyllene (٠,٩-٦,٤٪). النباتات التي تم حصادها في ٢١ فبراير أعطت زيت طيار به أعلى تركيز نسبي من chamazulene و geramacrene-D و لكن هذان المركبان انخفض تركيزهم تدريجيا مع مواعيد الحصاد المتأخرة. بينما تم الحصول على أعلى تركيز من β-pinene و limonene في النباتات التي تم حصادها في ١٥ مايو. هذه النتائج تشير إلى أن محصول الرؤوس الزهرية و محتوى النبات من الزيت الطيار بالإضافة إلى مكونات الزيت الطيار يمكن أن تتأثر بميعاد الحصاد و الظروف المناخية. هذه النتائج هامة لمنتجي نبات الآشيليا.