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STUDIES ON YARROW " *Achillea millefolium* L." PLANTS: II. EFFECT OF STORAGE PERIOD OF THE DRIED FLOWERS ON THE ESSENTIAL OIL PERCENTAGE AND COMPOSITION.

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ABSTRACT

Yarrow (*Achillea millefolium*, L.) plant, Fam. Asteraceae, locally known as "Thousand leaves", is an important medicinal and aromatic plant that contain high quality essential oil of deep blue color which is rich in chamazulene. A study was carried out during two successive seasons 2000/2001 and 2001/2002, at the Experimental Station of Medicinal and Aromatic plants, Fac. of Agric., Mansoura Univ. The research aimed to study the effect of length of the storage period on essential oil percentage of the dried flowers collected from two consequent harvests from spring and autumn plantings and the changes that took place during storage in the composition and the relative percentage of its constituents. The physical and chemical properties of the oil were determined and are included in this study.

The results showed that increasing the storage period of the dried flowers significantly decreased the essential oil percentage of the flowers by an average of 0.06% every two months. The planting date or the interaction between planting date and the storage period did not significantly affect the essential oil percentage of the dried flowers.

Gas Liquid Chromatography analysis of oil samples revealed ten identified compounds. Chamazulene was the major constituent of the oil forming from 18.42% to 47.32%. The other constituents were β-pinene, thujone, limonene,1-8 cineol, bisabolol, camphor, α _pinene, borneol, terpeinol, and caryophyllene. Increasing length of storage period decreased the percentage of (borneol, terpinol, 1-8 cineol,camphor, thujone and bisabolol) and increased the percentage of (α,β-pinene, limonene and caryophylene). While chamazulene percentage increased after two months of storage and decreased thereafter. Oil of autumn planting was higher in chamazulene, α- and β-pinene, but lower in limonene, caryophylene, 1-8 cineol, Bisabolol, Terpinol, camphor and borneol than spring. The oil of the 1st cut of both plantings contained higher percentages of α -pinene, camphor, borneol, thujone, terpinol and chamazulene, but lower β-pinene and bisabolol than the comparable ones of the 2nd cut.

INTRODUCTION

Medicinal and aromatic plants are natural therapy source to replace the chemical one. *Achillea millefolium*, L. (Asteraceae), commonly known as yarrow is a native to Europe and western Asia (Keville, 1999). The aerial parts of Achillea contain high quality essential oil of deep blue color, and its main constituent is chamazulene (Svoboda and Hampson, 2001). Achillea plants at the flowering stage contained maximum amount of oil and azulenes (Rohloff et al., 2000; Orav et al., 2001).

The essential oil of Achillea contains several physiologically active substances that reduce inflamation, help cold and flu, digestiv, antiseptic, antispasmodic and arresting hemorrhage, beside its effectiveness in lowering blood pressure (Chiej, 1984; Keville, 1999).

Length of the storage period of plant materials (Singh *et al.*, 1994), planting date (Guerrero and Johnson, 2000; Massoud, 1980) and date of

harvest (Mohamed, 1997; Bottcher *et al.*, 2000) affect the percentage and composition of their essential oil and the relative percentage of its constituents. These changes depend on condition of plant material, method and conditions of storage (i.e temperature and humidity), and the chemical composition of the essential oil (Paakonen *et al.*, 1990; Kotb and Eid, 1996).

The aim of this work was to study the effect of length of the storage period on the oil percentage of the dried flowers of Achillea that were collected from spring and autumn planting seasons. In addition, to study the differences and changes that take place during storage in the essential oil composition of Achillea dried flowers of two consequent harvests from spring and autumn plantings.

MATERIALS AND METHODS

This experiment was carried out during two successive seasons 2000/01 and 2001/02, on *Achillea millefolium*, L. plants (Fam. Asteraceae), at the Experimental Station of Medicinal and Aromatic Plants, Fac.of Agric., Mansoura Univ. The research aimed to study the effect of length of the storage period on essential oil percentage of the dried flowers collected from two consequent harvests from spring and autumn plantings and the changes take place during storage in the composition and the relative percentage of its constituents

Planting:

Yarrow (*Achillea millefolium*, L.) plants were cultivated using plant division at two different dates, autumn cultivation on (1st and 3rd November) in the first and second season respectively, and spring cultivation on (13th and 15th March) in the first and second season, respectively. The field was divided into 6 blocks (4 x 4 m each) containing 6 rows (each 3m long and rows were 60 cm apart). Planting was done at a distance of 50 cm between plants.

Harvesting:

At the flowering stage (when at least 50% of plants flowered) plants were cut at 20 cm height from the soil surface. Plants were cut twice in each season. In spring cultivation the first cut was done in the 1 of June, and the second cut in the 15 of August. In autumn cultivation, the first cut was done in the 15 of May and the second cut in the 15 of August. After harvesting, each plant was divided into herb and flowers that were dried in perforated paper bags under room temperature until constant weight.

Storage:

The dried samples (72 bags of dried flowers each harvest) were placed in paper bags for two storage periods (0, 2 and 4 months). A sample at each harvest was used as control.

Essential oil extraction and determination:

The essential oil was extracted from the dried samples (50g) by hydro-distillation using Clevenger apparatus according to methods described by the Egyptian pharmacopoeia (1984).

Physical and chemical properties of oil:

Samples of both seasons and harvests were mixed together and the essential oil was extracted to measure the general physical and chemical properties of the essential oil. These measurements were analysed at the laboratory of the Chemistry Deprt.,Fac.of Agric.,Mansoura Univ. The specific gravity, the refractive index and the optical rotation of the essential oil were determined according to the methods described by Guenther (1949). The acid number, saponification number and ester number of the oil were determined by applying the methods described in Guenther (1972).

Gas Liquid Chromatography (G.L.C.):

The G.L.C. analysis was carried out at the Central Laboratory of Cairo Univ. The relative retention time (RT) of each peak was compared with the reference authentic sample to identify the unknown samples. The quantitative estimation for each component was based on the peak area measurement by triangulation (Guenther and Joseph, 1978).

Statistical analysis:

A randomized complete block design with three replicates was used according to Steel and Torrie (1980). Data were subjected to the statistical analysis according to the analysis of variance procedure (ANOVA) using SAS computer software (SAS Institute, 1985). The treatment means were compared using the least significant difference (L.S.D) procedure as mentioned by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A. Physical and chemical properties of Achillea essential oil:

Identification of the physical properties (specific gravity, refractive index and optical rotation) and the chemical properties (acid number, saponification number and ester number) of the essential oil of Achillea are shown in Tab.1.

Table 1: Physical and chemical properties of the essential oil of Achillea essential oil.

Properties	Constant
Specific gravity	0.9394
Optical rotation	-8.5
Refractive index	1.390
Acid number	12.8
Saponification number	46.2
Ester number	33.4

B. Effects of storage and planting date on essential oil percentage:

1- Effect of storage period on essential oil %:

Increasing storage period of Achillea dried flowers significantly decreased the essential oil percentage of the flowers (Table 2). Each two months of storage decreased the essential oil of Achillea flowers by 0.06 % in the first season. In the second season, it was decreased by 0.05% after two months of storage and then, by 0.07% after another two months. Similarly,

Fehr (1980) mentioned that the oil of anise, carway and fennel fruits decreased by 1, 2.8 and 0.5 % when stored for a month respectively. On contrary, Shalaby *et al.* (1988) reported that the essential oil content of the stored samples of *Mentha arvensis* did not change throughout the storage period. However, the losses of essential oil from air dried plant material during storage depends on condition of material, method and conditions of storage, length of storage period and the chemical composition of the oil (Fehr, 1980; Kotb and Eid, 1996).

Table (2): Effect of storage period on Achillea essential oil % during two seasons (2000/ 01) and (2001/ 02).

Storage periods	1st season	2nd season
Control	0.62	0.65
2 months	0.56	0.60
4 months	0.50	0.53
L.S.D at 5 %	0.02	0.04

2- Interaction between storage period and planting date

Table (3) showed the effects storage on Achillea essential oil percentage of the two planting date. The data revealed that no significant differences in the percentage of the essential oil among treatments.

However, it was worth to note that the highest relative percentage of the essential oil was that of the spring planting extracted without storage of the flowers in both seasons of the experiment (0.64% in the first season and 0.73% in the second season). This data and those of the previous ones Table (2) showed that the main effect on the changes of the essential oil percentage was mainly due to the length of the storage period and was not affected by the season.

Table 3: Effect of storage period on the essential oil % of the two planting date during two seasons (2000/01) and (2001/02).

Planting date	Storage period	1st season	2nd season
Spring	Control	0.64	0.73
	2 months	0.59	0.63
	4 months	0.50	0.56
Autumn	Control	0.59	0.56
	2 months	0.54	0.57
	4 months	0.50	0.50
L.S.D at 5 %		N.S	N.S

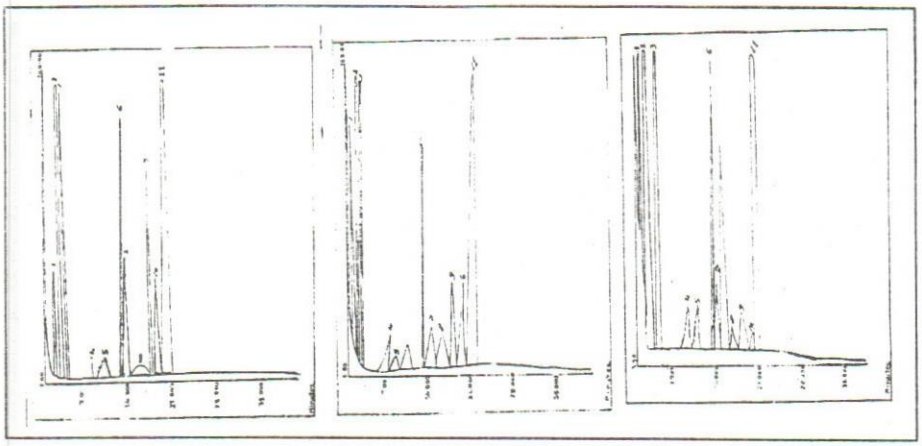
C. Effects of storage period, planting date and harvest time on the composition of the essential oil:

Gas liquid chromatography separation analysis (G.L.C.) was used to determine the composition of the essential oil of Achillea flowers produced from two planting seasons and collected at two consequent harvests from each season. The results of GLC analysis are shown in Tab.(4) and Fig.(1-4).

The identified compounds formed from 84.23% to 97.37% of the essential oil depending on time of planting, time of harvest and storage period.

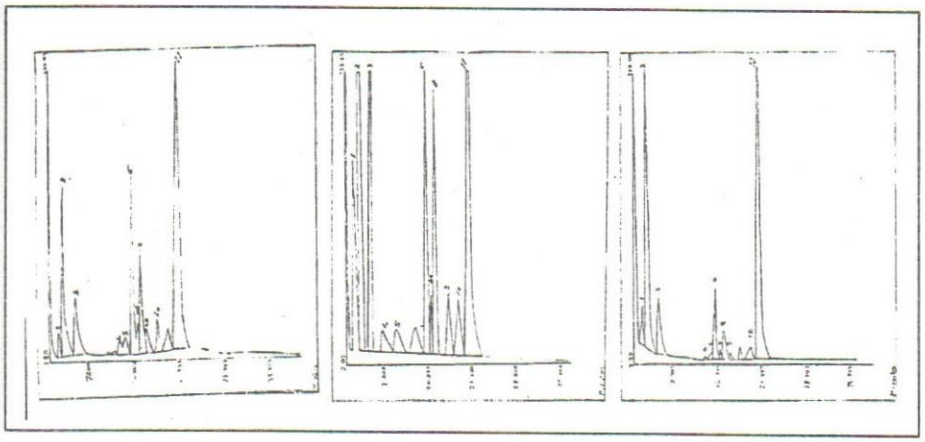
Table(4): Effect of planting date, time of harvest and storage period on the chemical composition of Achillea essential oil .

Storage period / Components	Spring 1st cut			Spring 2nd cut			Autumn 1st cut			Autumn 2nd cut		
	Cont.	2 mon.	4 mon.	Cont.	2 mon.	4 mon.	Cont.	2 mon.	4 mon.	Cont.	2 mon.	4 mon.
J. Pinene	2.12	4.03	6.1	1.49	3.98	7.66	3.97	7.07	9.87	2.14	4.04	5.56
a. Pinene	12.01	14.32	18.78	14.37	17.33	28.80	16.41	18.41	20.52	19.29	24.24	30.80
Limonene	8.70	10.40	16.00	7.57	9.06	16.43	5.85	8.21	15.41	8.06	8.94	12.46
Camphor	3.20	2.77	2.16	1.57	0.82	0.40	2.19	0.37	Traces	1.76	1.10	0.71
Borneol	2.41	1.99	1.60	1.89	0.84	Traces	2.21	1.31	Traces	1.57	Traces	Traces
Thujone	12.08	8.99	8.58	10.47	7.75	5.68	12.94	7.19	4.16	10.92	6.71	5.74
Terpeinol	3.85	2.46	1.18	1.85	1.54	0.56	1.81	1.76	1.55	0.84	Traces	Traces
Caryophyllene	0.12	1.44	2.10	3.01	4.16	7.73	1.24	1.56	2.68	0.54	1.17	1.49
1-8 Cineol	7.22	3.94	2.63	2.99	2.64	1.47	2.23	2.20	1.39	3.27	2.83	2.07
Bisabolol	3.88	3.08	0.64	4.17	3.34	1.10	1.14	1.81	0.95	4.85	1.04	1.03
Chamazulene	38.53	42.00	24.46	36.28	42.24	18.42	41.57	45.93	25.08	40.79	47.32	28.53
Known %	94.13	95.42	84.23	85.65	93.68	88.56	91.58	94.82	81.60	94.02	97.37	88.39
Unknown %	5.87	4.58	15.78	14.35	6.32	11.44	8.42	5.18	18.40	5.98	2.63	11.61



Control After 2 months After 4 months

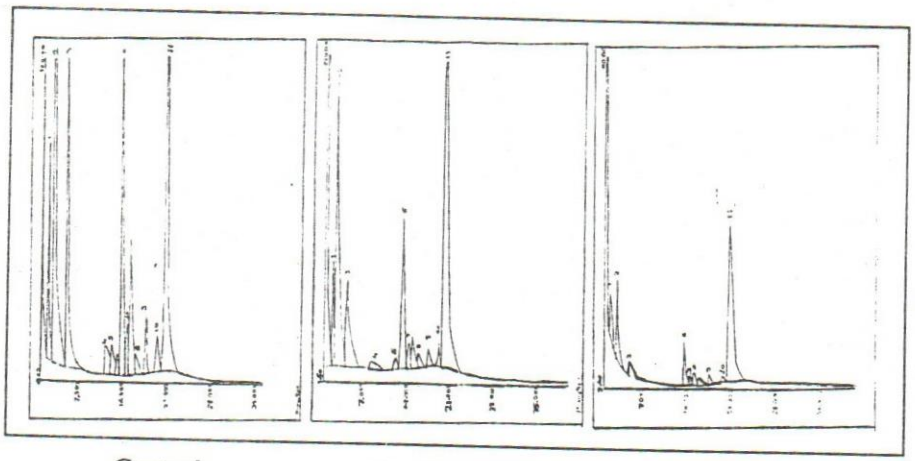
Fig (1): GLC of first harvest of Spring planting at different storage period in *Achillea millefolium*, L. plants.



Control After 2 months After 4 months

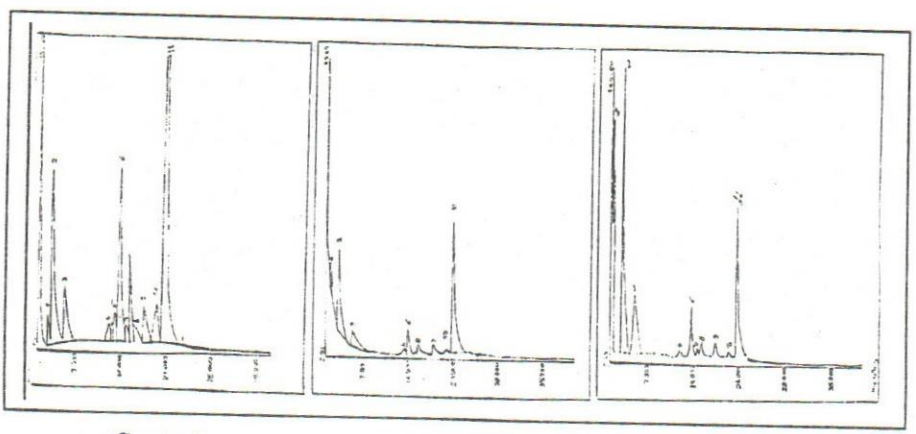
Fig (2): GLC of second harvest of Spring planting at different storage period in *Achillea millefolium*, L. plants.

1- α. Pinene 2- β. Pinene 3- Limonene 4- Camphor 5- Borneol 6- Thujone 7- Terpeineol 8-Caryophyllene 9-1.8. Cineol 10-α. Bisabolol 11-Chamazulene



Control After 2 months After 4 months

Fig (3): GLC of first harvest of Autumn planting at different storage period in *Achillea millefolium*, L. plants.



Control After 2 months After 4 months

Fig (4): GLC of second harvest of Autumn planting at different storage periods in *Achillea millefolium*, L. plants.

1- α. Pinene 2- β. Pinene 3- Limonene 4- Camphor 5- Borneol 6- Thujone 7- Terpeineol 8-Caryophyllene 9-1.8. Cineol 10-α. Bisabolol 11-Chamazulene

Generally speaking chamazulene the major constituent of the essential oil forming ranged from 18.42% to 47.32%, followed by β -pinene (12.01-30.80%), thujone(4.16-12.94 %) then limonene (5.85- 16.43 %). The other seven compounds were 1-8cineol (1.39-7.22%), bisabolol (0.64-4.85%), camphor (0.37-3.20%), α-pinene (1.49-9.87%), borneol (0.84-2.41%), terpeinol (0.56- 3.85%) and caryophyllene (0.12-7.73%).

These results are in accordance with previous reports, since chamazulene were reported to be the main constituent of Achillea essential oil (Svoboda and Hampson, 2001). On the other hand, Pino *et al.* (1998), found that caryophllene oxide (20%) was the major volatile constituent of *A. Millefolium*, L. grown in Cuba and Afsharypour *et al.* (1996) could not detect the presence of azulene in the essential oil of Iranian Achillea. The rest of the compounds previously mentioned were found in the essential oil of Achillea at variable concentrations (Kokkalou *et al.*, 1992; Shawl *et al.*, 2002).

Date of planting affected the relative percentages of the constituents. The oil samples from autumn planting were generally higher in Chamazulene, α-pinene and β-pinene concentrations, but were lower in caryophylene limonene, 1-8 cineol, Bisabolol, Terpinol, Camphor and Borneol percentages than the oil samples of spring planting, while Thujone percentages were relatively in different valeus between the two planting dates.

In this concern, Guerrero and Johnson (2000) reported that day length and temperature contributed to the difference in essential oil composition of Marjoram. In addition, light and temperature conditions affected Azulene content in Chamomile oil (Ghosh and Chatterjee, 1976). Also, Massoud (1980) found that autumn planting favored higher concentrations of β-pinene, cineol, but not α-pinene in the essential oil of *Saturia hortensies*, L.

Time of harvest also affected the relative concentrations of the essential oil constituents. In spring planting, the control samples of the oil extracted from the 1st cut contained higher percentages of α -pinene (2.12%), limonene (8.70%), camphor (3.20%), borneol (2.41%), thujone (12.08%), terpinol (3.85%), 1-8 cineol (7.22%) and chamazulone (38.53%) than the 2nd cut. On the other hand 2nd cut of spring planting had higher concentrations of β-pinene (14.37%), caryophyllene (3.01%) and bisabolol (4.17%) than the 1st cut.

In autumn planting, the control samples of the oil extracted from the 1st cut had higher percentages of α-pinene (3.97%), camphor (2.19%), borneol (2.21%), thujone (12.94%), terpinol (1.81%), caryophyllene (1.24%) and chamazulene (41.57%) than the 2nd cut of the same planting date. On the contrary, the control samples of autumn planting in the 2nd cut contained higher concentrations of β-pinene (19.29%), limonene (8.06%), bisabolol (4.85%) and 1-8 cineole (3.27%).

Thus it is obvious that the 1st cut of both plantings was higher in percentages of β-pinene, camphor, borneol, thujone, terpinol and chamazulene, but lower in concentrations of β-pinene and bisabolol than the comparable ones of the 2nd cut. Although Topalov and Zhelykov (1991), found that oil composition of *Mentha piperita* did not significantly differ with harvesting date, Khalil (1979) found that date of collection (harvesting)

affected azulene content in *Achillea millefolium*, L. Also, Mohamed (1997) on Thyme found that linalol, 1-8 cineol, bornyl acetate and terpinol were higher in the 2nd cut than the 1st cut and Bottcher *et al.* (2000) reported that the essential oil extracted from the 2nd cut of Melissa contained less quantity of linalool and caryophyllene.

The relative concentrations of each constituent changed with the length of the storage period. Increasing the storage period resulted in an increase in the percentages of α,β-pinene, limonene and caryophyllene, but a decrease in the oxygenated compounds (borneol, terpinol and 1-8 cineol) and the terpene hydrocarbons group compounds (camphor, thujone and bisabolol). On the other hand, chamazulene (the major constituent of the oil) increased after two months of storage and decreased thereafter. It is worth to note that the percentage of the unidentified compounds was highest after four months of storage. These changes during storage might be due to oxidation, disintegration, or transformation of different compounds. Similary, Singh *et al.* (1994) showed that the essential oil composition during storage changed with length of the storage period; some increased, some decreased, while others remained constant.

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دراسات على نبات الاشيليا (*Achillea millefolium* L.).

II – تأثير فترة تخزين الزهور الجافة على نسبة و تركيب الزيت العطرى

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نبات ال *Achillea millefolium* L. و يعرف محليا بالألف ورقة ينتمى للعائلة المركبة نبات طبى و عطرى هام يحتوى على زيت عطرى عالى الجودة ذو لون أزرق داكن غنى بمادة الكامازولين. و لقد تم عمل هذه الدراسة خلال موسمين زراعيين ناجحين فى عامى ٢٠٠٠/٢٠٠١ و ٢٠٠١/٢٠٠٢ بمزرعة النباتات الطبية و العطرية بكلية الزراعة جامعة المنصورة. و هدفت إلى دراسة تأثير طول فترة التخزين على نسبة الزيت العطرى فى الزهور الجافة التى تم جمعها من زراعتى الخريف و الربيع ، كما هدفت أيضا إلى دراسة الفرق فى تركيب الزيت العطرى فى الزهور الجافة التى تم جمعها من حشتين متتاليتين فى كل من زراعتى الخريف و الربيع و التغيرات الحادثة على نسبة مكونات الزيت خلال فترة التخزين.

أظهرت النتائج أن زيادة فترة تخزين الزهور الجافة أدت إلى نقص معنوى فى نسبة الزيت العطرى بنسبة ٠.٠٦ % كل شهرين و لم يؤثر موعد الزراعة أوتفاعله مع طول فترة التخزين معنويا على نسبة الزيت العطرى فى الازهار الجافة .

أظهر التحليل الكروماتوجرافى لعينات الزيت ١١ مركب و كان المركب الرئيسى له هو الكامازولين مكونا ١٨,٤٢% –٤٧,٣٢% من الزيت. وكانت المركبات الأخرى بالترتيب ألفا و بيتا –باينين ، ثيوجون ، ليمونين ، سينيول ، بيزابولول ، كامفور ، بورنيول ، تربينول ، كاريوفيللين. و لقد أدت زيادة طول فترة التخزين إلى زيادة ألفا و بيتا – باينين ، ليمونين ، كاريوفيللين و لكن أدت لإنخفاض نسبة المركبات المؤكسدة (بورنيول ، تربينول و سينيول) و المركبات الحلقية الهيدروكربونية (كامفور وثيوجون و بيزابولول) ولقد إرتفعت نسبة الكامازولين بعد شهرين من التخزين ثم إنخفضت بعد ذلك . و إحتوى الزيت الناتج من زراعة الخريف على نسب أعلى من الكامازولين، ألفا و بيتا – باينين و نسب أقل من الليمونين ، كاريوفيللين ، سينيول ، بيزابولول ، تربينول ، كامفور و بورنيول عن الزيت الناتج من زراعة الربيع. و إحتوى زيت الحشة الأولى فى كل من الربيع و الخريف على نسب أعلى من الكامازولين و ألفا باينين و كامفور و بورنيل و ثيوجون و تربينول و نسب أقل من بيتا باينين و بيزابولول مقارنة بالحشة الثانية.