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EFFECT OF DIFFERENT HARVESTING STAGES ON QUANTITY AND QUALITY OF HERB AND OIL YIELD OF EGYPTIAN THYME (*Thymus vulgaris* L.) PLANT.

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ABSTRACT

This study was carried out during the season of 2002 at Faculty of Agriculture, Ornamental Horticulture Department and Food Technology Research Institute, Agricultural Research Center (ARC) Giza, Egypt.

The seasonal variation of herb composition, essential oil content of Egyptian thyme (*Thymus vulgaris* L.), Physiochemical properties of the essential oil and analyzed by GC were examined. The results showed that at pre-flowering stage, thyme herb contained the highest content of moisture and total carbohydrates. The highest protein, ash and oil contents were obtained at full flowering stage. Whereas the highest content of fat as well as crude fiber were obtained at post flowering stage. The essential oil characterized by a highest content of thymol at full flowering stage .The specific gravity and refractive index of oil did not change appreciably due to the different harvesting stages. The essential oil at pre-flowering stage contained the highest p-cymene, linalool and carvacrol contents. A delay in harvesting from pre-flowering to post flowering stage decreased acid and ester values.

Keywords: *Thymus vulgaris* , Lamiaceae, essential oil composition, seasonal variation, physicochemical properties, chemical composition of thyme herb.

INTRODUCTION

Thyme (*Thymus vulgaris* L.)belongs to Family *Lamiaceae* (Labiatae). It is one of the medicinal plants employed throughout the Mediterranean region. It was used by the ancient Egyptians in the embalming process, Janulewicz (2001).

Thyme volatile phenolic oil has been reported to be among the top ten essential oils, showing antibacterial, antimycotic, antioxidant, natural food preservative, and mammalian age delay properties Letchamo *et al.*, (1995).

It has been known that agricultural practices have a great effect on both quality and quantity of essential metabolites. For this reason, it is necessary to determine the optimum harvesting time that affects plant growth and production Badi *et al.*, (2004). The time of harvesting is one of the most important agronomical factors and the seasonal variation has a significant effect on the yield and composition of thyme oil (McGimpsey *et al.*, 1994). Gill and Randhawa (1996) tested the effect of harvesting stages on the physicochemical properties of French basil (*Ocimum basilicum* L.). A delay in harvesting from vegetative to full flowering stage increased the optical rotation of herb oil, but decreased the ester value. Senatore (1996) studied the essential oil of the Italian thyme *(Thymus pulegioides)*, and found that sixty three compounds had been identified. Essential oils were characterized by a high content of gamma terpinene, p-cymene, thymol and carvacrol which varied from 57.3 to 62.5% of the total oil content. He added that essential oil yield and composition vary through out the vegetation time of the plant. The best time to harvest thyme for both essential oil yield and phenol content was during or immediately after the full bloom. Martins *et al.* (1999) recorded the seasonal variation in the yield and composition of the essential oil from collective samples of *Thymus zygis* L. The essential oil yield showed a maximum content at the flowering stage (0.9 -1.4%) and a minimum content during the dormancy period (about 0.15%). The composition of the essential oil also showed different patterns at different phases of vegetative cycle. At the flowering period, the essential oil was rich in thymol and geraniol. P-cymene showed a variation presenting a maximum at the minimum of thymol (post-flowering period). Aiming the use of the essential oil as a food ingredient the most interesting stage is the post-flowering period, the essential oil at this time being rich in thymol (about 21%), geranyl acetate (about 17%) and geraniol (about 13%), with P-cymene presenting lower levels. Hudaib *et al.* (2002) evaluated the volatile oils of (*Thymus vulgaris* L.) plants harvested at different periods during the plant vegetative and life cycles, the plants collected in June / July just before the end of the vegetative cycle, provided the best oil yield (1.2%) with also the highest percentage of the monoterpene phenols (thymol : 51.2% and carvacrol : 4%). This latter growth period can represent the best harvest time of young thyme plants in order to obtain an essential oil with better quality and quantity. Asllani and Toska (2003) studied the chemical composition of Albanian thyme oil (*Thymus vulgaris* L.). They found that, the highest quantity of thymol and carvacrol was presented in oils produced during the full flowering period.

MATERIALS AND METHODS

This work was conducted at the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University ,and Food Technology Research Institute, Agricultural Research Center (ARC), Giza, Egypt during 2002 season.

Thyme herb was obtained from a private farm in Beni Swif Governorate. The herb was harvested at three different harvesting stages (pre-flowering , full- flowering and post- flowering stages. The content of moisture, ash, crude protein, lipid, fiber, essential oil were determined according to A.O.A.C. (2000). The determination of total carbohydrates content was carried out according to Herbert *et al.* (1971).

The essential oil content of thyme herb was obtained by steam distillation by using clavenger's apparatus, the volatile oil was collected after 3hrs distillation according to British Pharmacopoeia (1988). The oil was dried by adding anhydrous sodium sulphate and filtrated. The bottles were held at -18 °C till analysis. Gas chromatographic analysis of thyme essential oil was performed using a Hewlett-Packard 5890 A series II instrument equipped with a flame ionization detector (FID) and a carbowax fused silica column (50m length, 0.25 mm. i.d. , film thickness 0.32 µm). The oven temperature was programmed from 60 °C to 200 °C at the rate of 3°C/min., helium (1 ml /min) was used as carrier gas; flow rates of air and hydrogen were 30 ml/min, split ratio 1:100 ;the temperature of injection part and detector were 150° C and 250° C , respectively . Percentages of peaks area were calculated with a Hewlett-Packard 3396 integrator.

Physical properties (specific gravity, optical rotation, refractive index) and chemical properties (acid value, ester value, and solubility in alcohol) of the oil were determined according to the Egyptian Pharmacopoeia (1984).

RESULTS AND DISCUSSION

Thyme herb composition :

The obtained data in Table (1) show that thyme plant had a highest moisture content at pre- flowering stage (13.00%) compared with the lowest moisture content at post- flowering stage (11.35%). The differences among moisture contents at different harvesting stages were statistically insignificant (Table 1). Delaying harvesting stages to post- flowering resulted in lower protein content in thyme plant than in full flowering stage. While the highest fat content (8.20%) were obtained in post flowering stage, followed by 7.22% at pre- flowering stage. Whereas, at full- flowering stage thyme plant content showed the lowest fat content compared with other harvesting stages. This may be attributed to the the highly consumed fats during the full flowering stage. whereas, at the post-flowering stage the fat may be accumulated and stored as a nutrient source for the different processes of the plant. Concerning carbohydrates content, the highest value was presented in thyme plant at pre- flowering stage (44.53%), then decreased to reach its lowest content (39.25%) of the plant. This reduction may be caused by the increasing at the post flowering stage of consumption rate through respiration and other processes than the photosynthetic rate of carbohydrates formation. The obtained data as shown in Table (1) indicated that fiber content increased with progressive of the life of thyme plants. While, thyme herb at full- flowering stage had a higher ash content (15.27%) compared with the two other stages (11.70 and 13.34%) for pre flowering and post flowering stages, respectively. These results agreed with those obtained with Kirk and Sawyer (1991), since they mentioned that the chemical composition of thyme (*Thymus valgaris*) herb was as follows; moisture 12%, total ash 14% (dry matter basis), acid insoluble ash 5% ,crude fiber 30%, volatile oil 1%.

Table (1) Chemical composition of thyme herb at different harvesting stages (%):

Constituent	Pre- flowering	Full flowering	Post flowering
Moisture	13.00	12.55	11.35
Crude protein	9.12	9.64	8.97
Fat	7.22	6.60	8.20
Total carbohydrates	44.53	40.32	39.25
Fiber	16.18	18.77	22.75
Ash	11.70	15.27	13.34
Essential oil	0.85	1.49	1.20

Ensminger *et al.* (1995) reported that the chemical composition of thyme herb was as follows: moisture 7.8 % ,protein 9.1 % , fat 7.4 % , carbohydrates 63.9 % and fiber 18.6 % . Hamza *et al.* (2001) reported that the chemical composition of thyme was as follows: Moisture 7.30 % , protein 9.15 % , fat 7.44 % ,ash 11.76 % ,carbohydrates 64.35 % and crude fiber 18.70 % (on dry weight basis).

The oil content of *Thymus vulgaris* L. collected at three harvesting stages, are shown in Figure 1. Oil content at full flowering stage (1.49%) was significantly higher than that obtained at pre- flowering and post flowering stages (0.85 and 1.20%), respectively. At early pre- flowering stage the essential oil content was significantly lower than that obtained at other harvesting stages. Similar results were obtained by Senatore (1996) , Martins *et al.* (1999) and Hudaib *et al.* (2002) on thyme plant.

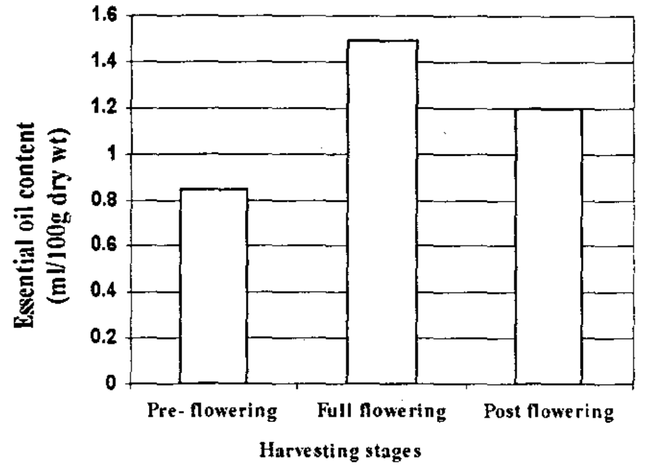


Fig.(1) Seasonal variation of the essential oil content of thyme herb.

Physicochemical properties of oil :

The quality and consequently the value and application of essential oil are highly correlated with their physicochemical characteristics. For this reason the physical and chemical properties are usually determined in order to assess the value and application of such products.

Delaying harvesting stages to post flowering increased specific gravity in thyme oil than other stages, but the differences were not significant. While various harvesting stages did not change appreciably the refractive index of thyme herb oil (Table 2). The obtained values of optical rotation for thyme essential oil were +10.52, +10.35 and +10.43 for pre- flowering, full flowering and post flowering, respectively. The optical rotation of herb oil decreased with a delay in harvesting stage from pre flowering to full flowering stage, while a further delay in harvesting, increased the optical rotation . The solubility of many essential oils in diluted ethyl alcohol is in many cases, considered a good criterion for the assessment of its quality (Guenther, 1961). Furthermore, adulteration of many essential oils could be detected very easily by the determination of their solubility (Poucher, 1955). The solubility in alcohol of thyme oil increased with a delay in harvesting to full flowering , but at post flowering stage the solubility of thyme oil decreased.

The acid value of herb oil decreased with a delay in harvesting to post flowering stage. A similar trend was observed in ester value of herb oil.

Table (2) Physicochemical properties of thyme essential oil extracted at different harvesting stages:

Properties	Pre flowering	Full flowering	Post flowering
Specific gravity*	0.875	0.915	0.954
Refractive index*	1.5041	1.5055	1.5043
Optical rotation*	+10.52	+10.35	+10.43
Solubility in alcohol	1gm/2.0ml	1gm/2.5ml	1.0gm/2.2ml
Acid value	1.14	1.2	0.98
Ester number	10.49	9.77	8.41

*at 20°C

GLC analysis of oil :

The chemical composition of thyme essential oil extracted at different harvesting stages during plant life cycle are given in Table (3) about (22, 14, and 12) volatile components were identified in thyme essential oil extracted at (pre flowering–full flowering– post flowering) stages, respectively.

Table (3) Chemical composition (%) of thyme essential oil extracted at different harvesting stages.

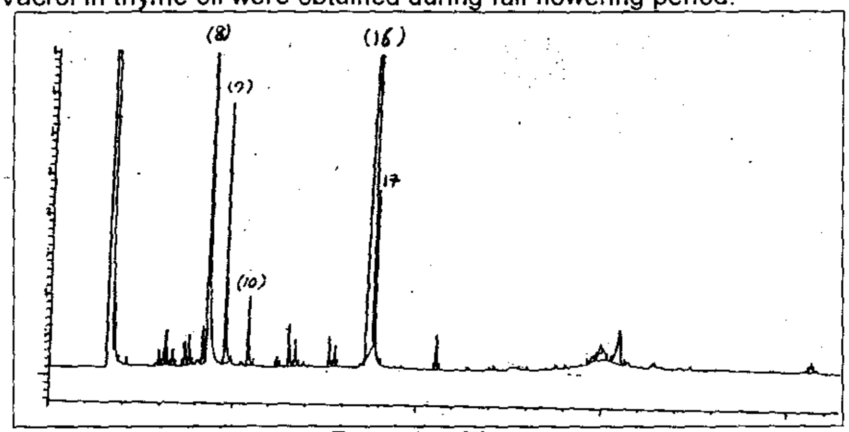
NO.	Component	Pre- flowering	Full flowering	Post flowering
1	α - thujene	0.58	0.79	0.98
2	α - pinene	0.45	0.35	0.60
3	Camphene	1.13	1.16	1.42
4	Sabinene	0.68	0.78	0.97
5	β-pinene	1.09	----	----
6	Myrcene	1.00	1.14	1.56
7	ά – terpinene	1.37	1.32	1.48
8	ρ- cymene	20.4	7.79	9.61
9	δ- terpinene	8.02	13.04	15.59
10	Linalool	2.10	----	----
11	Camphor	0.31	----	----
12	Borneol	1.31	----	----
13	Thymol methyl ester	0.77	0.35	0.36
14	Carvacrol methyl ester	0.78	----	----
15	Thymoquinene	0.51	1. 35	0.64
16	Thymol	48.13	69.66	66.3
17	Carvacrol	3.58	----	----
18	Thymol acetate	0.96	0.51	0.26
19	ά- copaene	0.51	----	----
20	β- caryophyllene	2.12	----	----
21	β- caryophyllene oxide	3.56	----	----
22	D- germacrene	0.60	1.37	----
23	β -bourbonene	----	0.21	----

In thyme essential oil extracted at pre flowering stage, the major components were thymol (48.13%), P-cymene (20.40%), δ-terpinene (8.02%), carvacrol (3.58%), β-caryophyllene oxide (3.56%), β- caryophyllene (2.12%) and linalool (2.10%).

Also, from the same Table, it could be noticed that the main identified compounds percentage of thyme oil extracted at full flowering stage were thymol (69.66%), δ-terpinene (13.04%), P–cymene (7.79%), thymoquinene (1.35%) and D–germacrene (1.37%).

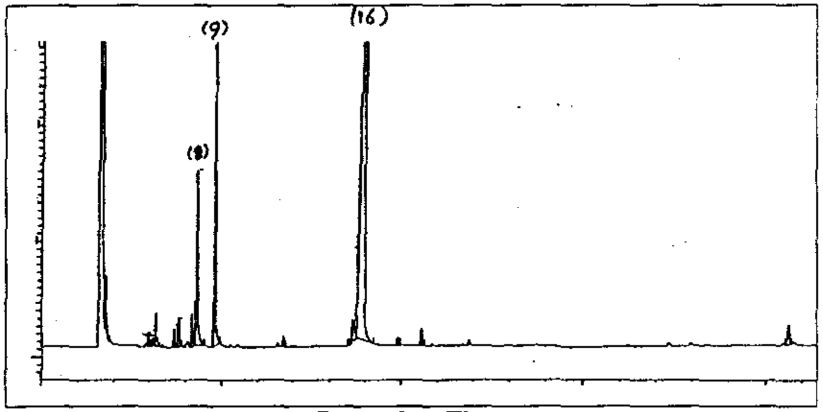
Furthermore, from the same data, it could observed that, some of the components disappeared such as: β–pinene, linalool, camphor, carvacrol, carvacrol methyl ester, α–ccpaene, β–caryophyllene, and β–caryophyllene oxide. On the other hand, there were an occurrence for a new component: β–bourbonene (0.21%). In addition, in post harvesting stage there were components identified, the portion of the main components were thymol (66.33%), δ–terpinene (15.59%), P–cymene (9.61%), myrecen (1.56%) and α–terpinene (1.48%). In comparison with the chemical composition of thyme essential oil harvesting at pre- flowering and full flowering stages, we can notice the disappearance of D–germacrene in thyme oil extracted in post flowering stage. In general, it is evident that thymol is the main component in thyme oil in the three harvesting stages.

Concerning the effect of different stages of plant growth of thyme essential oil on its chemical composition, it could be concluded that, the number and percentage of thyme oil compounds varied in different extraction periods. The result also indicated that full flowering stage is the best harvest time of thyme plants in order to obtain better quality and quantity. Highest quantity of thymol was present in oils produced during the full flowering period and after it. These results are in agreement with those obtained by Senatore (1996) on Italian thyme (*Thymus pulegioides*) , Martins *et al.* (1999) on *Thymus zygis* , Hudaib *et al.* (2002) and Asllani and Toska (2003) on *Thymus vulgaris* . They showed that the highest percentage of thymol and carvacrol in thyme oil were obtained during full flowering period.



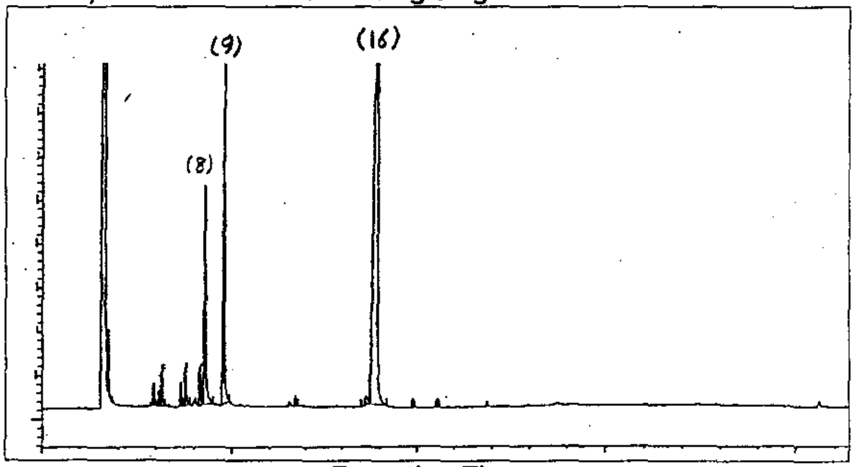
Retention Time

Fig.(2): Chromatogram of essential oil of thyme plant (*Thymus vulgaris* L.) distilled at pre-flowering stage.



Retention Time

Fig.(3): Chromatogram of essential oil of thyme plant (*Thymus vulgaris* L.) distilled at full flowering stage.



Retention Time

Fig.(4): Chromatogram of essential oil of thyme plant (*Thymus vulgaris* L.) distilled at post flowering stage.

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تأثير مواعيد الحصاد المختلفة علي كمية وجودة العشب و الزيت الطيار في نبات الزعتر

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**** معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة**

اجري هذا البحث خلال موسم ٢٠٠٢ بقسم نباتات الزينة – كلية الزراعة – جامعة القاهرة ومعهد بحوث تكنولوجيا الأغذية – معهد البحوث الزراعية – الجيزة. تم دراسة تأثير الإختلافات الموسمية للتركيب الكيماوي للعشب، ومحتوي الزيت الطيار، والصفات الطبيعية والكيماوية وكذلك التركيب الكيماوي للزيت الطيار في نبات الزعتر. ويمكن تلخيص أهم النتائج فيما يلي : محتوي العشب من الرطوبة والكربوهيدرات الكلية كان أعلي ما يمكن خلال مرحلة النمو الخضري (قبل التزهير). بينما تم الحصول علي أعلي محتوي من البروتين والرماد ونسبة الزيت الطيار خلال مرحلة اكتمال التزهير. في حين أن أعلي محتوي للدهون والألياف في العشب كان خلال مرحلة ما بعد التزهير . محتوي الزيت الطيار من مركب الثيمول كان أعلي ما يمكن خلال مرحلة اكتمال التزهير. لم يحدث تغيير في الكثافة النوعية ومعامل الانكسار للزيت الطيار خلال مواعيد الحصاد المختلفة . محتوي الزيت الطيار خلال مرحلة النمو الخضري (قبل التزهير) من البارا سيمين و الليناول و الكارفاكرول كان أعلي مايمكن .تأخير ميعاد الحصاد الي مرحلة ما بعد التزهير أدي الي نقص في رقم الحامض ورقم الاستر للزيت الطيار.