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# EFFECT OF PACKING MATERIALS AND STORAGE PERIODS ON PIGMENTS IN LEAVES, OIL CONTENT AND ITS CONSTITUENTS OF THYME *(THYMUS VULGARIS*, L.)

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## ABSTRACT

An experiment was conducted to study the effect of four packing materials namely, paper, cotton cloth, textile polyethylene and gunny bags on thyme leaves stored 18 months for periods up to samples were taken and analyzed every three months. The results indicated that the longest period for safe storage was attained in all cases of the paper bags (18 months) followed by textile polyethylene and gunny bags (12 months). The worst was that of cotton cloth bags during the storage periods in most cases, also gunny bags revealed less value at 18 months. The moisture content was increased over control in all packing materials used. At all periods of storage the highest value of volatile oil percentage was recorded with paper bags till 18 months while the lowest one was recorded with gunny bags after 18 months storage. Backing material differs in its effect on oil percentage through the storage period. The lowest oil percentage was of those served with gunny bag at 18 month period. Significant decreases in oil percentages were recorded, due to storage period, regardless packing materials, also paper bags were the most effective for reducing this effect.

The phenolic compounds of thyme oil (thymol and carvacrol) were slightly decreased in leaves stored in textile polyethylene bags for 6 months comparing with the control. Thymol and carvacrol contents were decreased by 6.86 and 13.4%, respectively, while the decrease of thymol reached 52.86 and 63.56% for paper and gunny bags, respectively, under control at the end of storage periods. The most favourable packing material was of paper bags, which suited longer period of storage with less decrease in both volatile oil and thymol percentages and for 12 months.

## INTRODUCTION

Thyme (*Thymus vulgaris*, L.) is a member of Lamiaceae family, which is distributed in the area of Mediterranean region and it grows wild in Sinai (Jackson and Hay, 1994). It is a strong aromatic perennial evergreen subshrub up to 45 cm in high with much branched upright stem and small flowers united in spikes at the top of the branches (Lawless, 1977 and Chiej, 1988). Thyme consists of whole leaves and flowers separated from the previously dried stems, it contains not less than 12mg/kg of essential oil and not less than 0.5% phenols expressed as thymol (British Pharmacopoeia, 2003).

The volatile oil produced from the leaves and flowering tops contains the most important constituents e.g. the phenols thymol and carvacrol; terpenoids; glycosides of phenolic monoterpenoids; eugenol and aliphatic alcohols; flavonoids thymonin, cirsilineol and 8- methoxycirsilineol; biphenyl compounds of monoterpenoids; caffeic and rosmarinic acid and saponins (ESCOP, 1997 and Byeoung *et al.*, 2005).

The thyme oil is used both internally and externally to treat a great variety of diseases. It is polyvalent remedy and is used for symptoms of bronchitis and whooping cauch and catarrha of the upper respiratory tracts. It has also been used to improve digestion and to treat pertussis, stomatitis and halitosis (ESCOP, 1997 and Wichtl and Bisset, 1994). The essential oil of thyme is used as a flavoring agent in the food industry, in the manufacture of perfumes and cosmetics and for medicinal purposes such as preparations of antispasmodic and tonic products (Figueiredo *et al.*, 1993). Also, thyme oil is extensively used in pharmaceutical industry as antiseptic and antimicrobial (Deans *et al.*, 1993; Dorman and Deans, 2000; Guynot *et al.*, 2003; Plaza *et al.*, 2004 and Badi *et al.* 2004) Solomakos and Govaris, 2004 reported that thyme volatile phenolic oil has been used as antibacterial, antimycotic, antioxidative, natural food preservative and mammalian age delaying properties.

Also, antiinflamatory effect is due to the effects of thyme oil and thymol compound on fungi (Geordani *et al.*, 2004 and Zambonelli, *et al.*, 2004).

Vigo *et al.* (2004) used *Thymus vulgaris* in traditional medicine in the treatment of bronchitis asthma and other respiratory diseases. The aroma constituents of thyme contains thymol and carvacrol, which are very effective as antimicrobial and antioxidant (Solomakos and Govaris, 2004 and Seung Joo *et al.*, 2005).

El-Zbieta and Koztowski (1962) found that the oil content of chamomile, mint, melissa and salvia leaves was decreased by a considerable higher rate when the samples were stored in paper bags. Fehr (1980) on fennel, anise and caraway found that the oil of anise fruits was decreased during the storage period by the rate of 1% per month. However, in caraway fruits the rate was 2.8% per month and in fennel samples, it changed from 0.01 to 0.15 % per month. Ahmed and Eid (1996) on chamomile, found that the oil percentage decreased gradually till the end of the storage period. The decrease in oil percentage was pronounced in plastic bags than in card box and glass jar. Venskutonis *et al.* (1996) cleared that storage of thyme in polyethylene bags was not aroma-tight as some losses occurred due to evaporation of volatile compounds. Abd-El-Latif *et al.* (2001) stated that total chlorophylls were decreased by increasing time of storage of dried herbs at room temperature up to three months. El-Kady (2003) found that during storage of marjoram up to nine months, the chlorophyll content degradation was gradual. Moreover, the moisture content of dried marjoram was gradually increased during storage period. This was due probably to the permeability to gases of Jute and polyethylene pouches including the atmospheric vapour including a slight increase in moisture content of the dried herb. He also, stated that the essential oil content of marjoram decreased gradually during storage up to nine months at room temperature. Misharina *et al.* (2003) studied the composition of volatile compounds of the essential oil of marjoram plants and its stability during storage. They declared that storage in the dark for one year was associated with insignificant changes in the composition of the essential oil. Also storage in the light produced considerable changes in the composition of the oil, due to chemical transformation of terpenoids, which may be more active in light.

Regarding the abovementioned information, this work aimed to attain the best packing material in storing thyme leaves, as well as the longest storage period at room temperature.

## MATERIALS AND METHODS

This work was carried out at the Department of Medicinal and Aromatic Plants, A.R.C. Dokki, to study the effect of packing material and storage periods on the pigments of dried leaves, essential oil content and quality during 18 months starting from st. July (2004) till 1st January (2006)..

The packages used in the experiment were paper, cotton cloth, textile polyethylene and gunny bags. All bags were 15 x 25 cm. Each package type was filled with 250 gm of air dried thyme leaves which were obtained from the Farm of Medicinal Plants Section Shark El-Buhayrat, El-Esmailia, and air dried for one month after being harvest.

The packages were stored in a dry place at room temperature (18-25°C) for 0, 3, 6, 9, 12, 15 and 18 months, and three bags were used for each package type, at the previously mentioned intervals of each storage period.

Data of the present study were statistically analyzed according to Snedecor and Cochran (1989), as split blocks design. The main blocks were package type and sub blocks were storage periods. Means were compared using L.S.D. values at 5% level.

The following data were recorded:

1- Volatile oil percentage using Clevenger apparatus according to Gunther (1961).
2- Moisture content using electric drying oven with a fan according to A.O.A.C. (1965), the moisture content was determined at the beginning and at the end of storage period used in the first and at the end of experiment .
3- Determination of chlorophylls a, b and carotenoids (mg/g.d.w.) were determined according to Saric *et al.* (1965).

4- Constituents separating of the hydrodistilled volatile oil obtained from both the starting, 6, 12 and 18 months (terminal samples) were carried out using G.L.C. technique Helweet pock was chromatograph column : Wcot fused silica 25 Mx 0.25 MMID coativo cp-sil 50 B DF = 0.12 (varron). Temp. programming : initial temp. 80 °C, rate (1) 10 °C/min. Final temp. 135 °C, rate (2) 5o °C/min. Final temp. 190 °C, rate (3) 10 °C /min. Final temp. 270 °C. Injector : Mode split (1 : 100), temp. 250 °C , flow 1.5 Detector : Temp 275 °C; mode constant flow.

## RESULTS AND DISCUSSION

**Effect of storage period and packing materials on chemical composition of thyme leaves:-**

**- Moisture content:-**

Data in Table (1) showed that moisture percentage of thyme herb was 7.65% at zero time of storage. Concerning the effect of storage period on moisture content, it could be noticed that the moisture content was increased in thyme leaves till the end of storage (18 months). The rates of increments were 11.63, 14.50, 15.82 and 16.47% over control for paper, cotton cloth, textile polyethylene and gunny bags, respectively. It could be concluded that the highest moisture content in thyme leaves was exhibited by packing in gunny bags, while the lowest value was given by paper bags. These results coincided with those obtained by Hassan *et al.* (1989) on peppermint, El-Deeb *et al.* (1993) on coriander and El-Kady (2003) on marjonam and rosemary. This fluctuated trend may be attributed to the perforation in the used material which permits the exchange of the air inside and outside the bags. This exchange is influenced by the low relative humidity and higher temperature prevailing during summer and winter months.

**Table (1): Effect of packing material and storage period on moisture percentage of *Thymus vulgaris*, L. leaves.**

| Parameter | Mean of moisture % | | |
|---|---|---|---|
| Packing material | Starting storage | End of storage | Decrease % |
| Paper bags | 7.65 | 8.54 | 11.63 |
| Cotton cloth bags | - | 8.76 | 14.50 |
| Textile polyethylene bags | - | 8.86 | 15.82 |
| Gunny bags | - | 8.91 | 16.47 |

**- Pigments content:-**

**- Chlorophyll "a and b" contents (mg/g.d.w.):**

Data in Table (2) demonstrated that both chlorophyll a and b contents ranged from 1.54 to 0.70 (mg/g d.w.) respectively, for thyme leaves at zero time.

Concerning the effect of storage period on chlorophyll contents (a and b), it could be noticed from Table (2) that there was a significant gradual decrease in chlorophylls content of thyme leaves during storage periods up to 18 months with all types of packages used. The percentage of decrease below control was 38.31 and 50% for chlorophyll a and b respectively, during storage periods up to 18 months. This is probably due to the conversion of chlorophyll to pheophytin. These results were parallel to those obtained by Hassan *et al.* (1989); Abd-El-Latif (2001) and El-Kady (2003) who found that during storage of marjoram up to nine months at room temperature, the chlorophyll content degradation was gradual. The loss increased with increasing period of storage.

Concerning packing materials and its effect on chlorophyll "a and b" contents, the results showed that paper bags gave the highest values of chlorophyll contents "a & b" (1.22 and 0.56 mg/g d.w.), while gunny bags came at least (1.12 and 0.53 mg/d.w), respectively.

**- Caroteneoids content (mg/g d.w.):**

Data in Table (2) showed that the carotencoids content ranged from (0.79 mg/g d.w.) at zero time to (0.61 mg/g d.w.) for thyme leaves at the end of storage period (18 months). It could be noticed that there was a gradual significant decrease in caroteneoids content of thyme leaves during storage periods up to 18 months. The percentage of decrement reached 22.78% lower than control (zero time).

It could be noticed that nearly a similar trend as previously obtained with chlorophyll was found with caroteneoids since paper bags was superior over all other types of packages, while gunny bags was the least in this concern. Since paper bage completely prevent the effect of light or air moisture while gunny bag gave more penetration for light and air moisture, which affects the sensitive molecular structure of carotene badly. This results were in agreement with those obtained by El-Kady (2003) on marjoram.

**- Essential oil content:**

Data in Table (3) illustrate the effect of storage periods and packing materials on essential oil content of thyme leaves. It could be observed that there was a gradual significant decrease in volatile oil contents of dried leaves during the storage periods up to 18 months, as reached from 1.5 to 0.43% This decrease represents 71.33% less than control (zero time). This is probably due to the volatility of oil during storage period at room temperature.

In concern to packing materials effects on the volatile oil content results showed that paper bags gave the lowest reduction in content. The reduction of volatile oil percentage was 38.67% less than control while in gunny bags, the reduction was 46% less than the control. This is mainly due to the structure of gunny bags, previously mentioned. This results were in agreement with those obtained by El-Deeb *et al.* (1993) on coriander, Venskutonis *et al.* (1996) on thyme. El-Kady (2003) stated that during storage up to nine months at room temperature, the essential oil content of *marjoram* was decreased gradually. This result was probably due to volatilization and essential oil emission. Ahmed and Eid (1996) on chamomile, concluded that the oil percentage was decreased by about 50% after 12 months from the beginning of storage.

**Table (2): Effect of packing material and storage period on chlorophyll a, b and carotenoid pigments content (mg/g dry weight basic) of *Thymus vulgaris*, L. leaves during 18 months of storage.**

| Storage periods | Packing materials | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bags | | | Cotton cloth bags | | | Tixtle polyethylene bags | | | Gunny bags | | | Means | | |
| | Chloro. a | Chloro. b | Caro. c | Chloro. a | Chloro. b | Caro. c | Chloro. a | Chloro. b | Caro. c | Chloro. a | Chloro. b | Caro. c | Chloro. a | Chloro. b | Caro. c |
| Zero time (7/2004) | 1.54 | 0.70 | 0.79 | 1.54 | 0.70 | 0.79 | 1.54 | 0.70 | 0.79 | 1.54 | 0.70 | 0.79 | **1.54** | **0.70** | **0.79** |
| 3 months | 1.25 | 0.71 | 0.90 | 1.28 | 0.70 | 0.89 | 1.31 | 0.71 | 0.87 | 1.28 | 0.68 | 0.91 | **1.28** | **0.70** | **0.89** |
| 6 months | 1.25 | 0.61 | 0.86 | 1.24 | 0.68 | 0.89 | 1.32 | 0.63 | 0.82 | 1.19 | 0.64 | 0.84 | **1.25** | **0.64** | **0.85** |
| 9 months | 1.21 | 0.56 | 0.84 | 1.12 | 0.52 | 0.85 | 1.21 | 0.51 | 0.76 | 1.09 | 0.50 | 0.68 | **1.16** | **0.52** | **0.78** |
| 12 months | 1.15 | 0.46 | 0.68 | 1.05 | 0.50 | 0.62 | 1.20 | 0.48 | 0.62 | 0.98 | 0.47 | 0.60 | **1.10** | **0.48** | **0.63** |
| 15 months | 1.12 | 0.43 | 0.67 | 0.99 | 0.42 | 0.61 | 1.04 | 0.42 | 0.61 | 0.92 | 0.42 | 0.59 | **1.02** | **0.42** | **0.62** |
| 18 months | 1.04 | 0.36 | 0.67 | 0.99 | 0.37 | 0.58 | 0.89 | 0.36 | 0.61 | 0.86 | 0.31 | 0.58 | **0.95** | **0.35** | **0.61** |
| Means | 1.22 | 0.56 | 0.78 | 1.17 | 0.55 | 0.72 | 1.21 | 0.55 | 0.73 | 1.12 | 0.53 | 0.67 | - | - | - |

L.S.D. at 5% for packing material of chloro. "a" 0.051 "b" 0.026 caro. "c" N.S.
L.S.D. at 5% for storage periods of chloro. "a" 0.037 "b" 0.021 caro. "c" 0.037.
L.S.D. at 5% for interaction between storage periods and packing material ofchloro. "a" 0.117 "b" 0.052 caro. "c" N.S.
chloro. = chlorophyll
caro. = carotenoid

**Table (3): Effect of packing material and storage period on volatile oil content of *Thymus vulgaris*, L. leaves during 18 months of storage.**

| Storage periods / Packing mterial | Volatile oil % Storage periods | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | Starting period | 3 months | 6 months | 9 months | 12 months | 15 months | 18 months | |
| Starting | 1.50 | - | - | - | - | - | - | 1.50 |
| Paper bags | - | 1.03 | 0.80 | 0.85 | 0.82 | 0.72 | 0.66 | 0.92 |
| Cotton cloth bags | - | 0.87 | 0.84 | 0.78 | 0.70 | 0.68 | 0.50 | 0.84 |
| Textile polyethylene bags | - | 1.05 | 0.86 | 0.83 | 0.80 | 0.60 | 0.36 | 0.85 |
| Gunny bags | - | 0.90 | 0.84 | 0.82 | 0.79 | 0.63 | 0.20 | 0.81 |
| Mean | 1.50 | 0.96 | 0.86 | 0.82 | 0.78 | 0.66 | 0.43 | |

L.S.D. at 5% for storage period 0.086.
L.S.D. at 5% for packing material N.S.
L.S.D. at 5% for interaction N.S.

- **Essential oil constituents:**

Data shown in Table (4) and Figs (1-4) illustrate the effect of storage periods and packing materials on phenolic volatile oil compounds, which are thymol and carvacrol as the major compounds of the thyme oil. It was noticed that thymol and carvacrol components at zero time were 41.85 and 3.21%, respectively.

It was noticed that there was a graduall decrease in these components during the different storage periods. Paper bags gave the lowest reduction, of 29.94 and 31% less than zero time for thymol and carvacrol, respectively, during storage period till 12 months, but there were some fluctuations in the trend, in between, so we can conclude that there was a clear trend for the effect of storage period on the essential oil components of thyme.

Taking the effect of packing material into consideration also, it could be noticed that paper bags exhibited the best backing which is suitable for longer storage period (18 months).

Textile polyethylene bags exhibited the best packing material in maintenance of the highest percentage of main components (38.98% thymol) after 6 months of storage followed by gunny bags (34.92%) but, cotton cloth bags gave the lowest percentage (29.25%), while the worst was the cotton cloth bags and textile polyethylene bags after 18 months of storage. These results are in accordance with Misharina *et al.* (2003) on marjoram, they declared that storage in the dark for one year was associated with insignificant changes in the composition of the essential oil, and its organoleptic characteristics remained largely unaffected. Also Eid (1999), reported that using polyethylene bags was the best way for packing caraway seeds for maintaining its essential oil content.

**Table (4): Effect of packing material and storage period on volatile oil constituents of *Thymus vulgaris*, L. leaves during 18 months of storage (7-2004 – 1/2006).**

| Peak. No. | Compound | RRT | Starting period | 7/2004-1/2005 | | | | 1/2005-7/2005 | | | | 7/2005-1/2006 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | P | C | T | G | P | C | T | G | P | C | T | G |
| 1 | α-pinene | 0.23 | 2.01 | 41.97 | 17.49 | 24.76 | 21.58 | 38.74 | 70.93 | 64.80 | 67.55 | 58.99 | 94.03 | 87.37 | 73.41 |
| 2 | p- cymene | 0.53 | 23.48 | 9.07 | 24.95 | 14.97 | 19.82 | 14.82 | 6.06 | 8.03 | 6.85 | 9.70 | 0.87 | 2.37 | 4.14 |
| 3 | δ-terpinene | 0.59 | 11.58 | 5.51 | 9.94 | 6.91 | 7.14 | 5.43 | 1.73 | 2.83 | 1.95 | 0.95 | 0.35 | 0.40 | 2.44 |
| 4 | Camphene | 0.63 | 0.80 | 0.55 | 0.50 | 0.58 | 0.52 | 0.45 | 0.15 | 0.35 | - | 0.31 | - | 0.25 | 0.25 |
| 5 | Borneol | 0.79 | 1.77 | 1.15 | 0.74 | 0.93 | 0.85 | 0.61 | 0.34 | 0.51 | - | 0.33 | - | 0.25 | 0.33 |
| 6 | Thymol | 1.0 | 41.85 | 33.93 | 29.25 | 38.98 | 34.92 | 29.32 | 15.77 | 17.43 | 19.58 | 19.73 | 4.43 | 7.64 | 15.25 |
| 7 | Carvacrol | 1.01 | 3.21 | 2.42 | 2.16 | 2.78 | 2.54 | 2.21 | 1.19 | 1.32 | 1.95 | 1.64 | 0.32 | 0.58 | 1.11 |
| 8 | Caryophyllene | 1.20 | 1.68 | 0.89 | 2.58 | 1.8 | 2.07 | 1.52 | 0.44 | 0.26 | - | 0.89 | - | 0.21 | 0.78 |

P = Paper bags C = Cotton cloth bags T = Textile polyethylene bags G = Gunny bags.

It could be concluded from these data, that thyme can be store with the least decrease in its content of oil or phenolic compounds for three months regardless packing material. The decrease in oil content due to storage periods reached 36.0, 42.7 and 48.0% for 3, 6 and 12 months, respectively, while after 18 months, the reduction reached 71.3% (more than half the original time).

Moreover, the best packing material which resulted in the least reduction in oil percentages or phenolic components (thymol and carvacrol) percentages as compared with the starting time was paper bags along all storage periods.

The longest safe period and best packing material for store thyme was one year using paper bags.

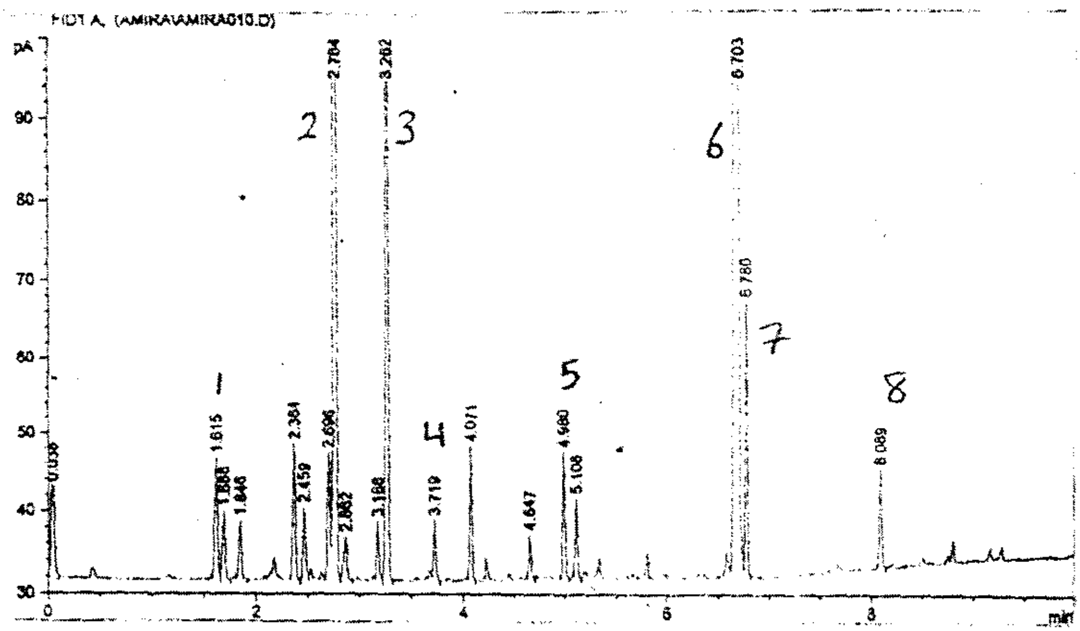


**Fig. (1): Chromatorgam of thyme volatile oil distilled from leaves of plants at zero time (control).**

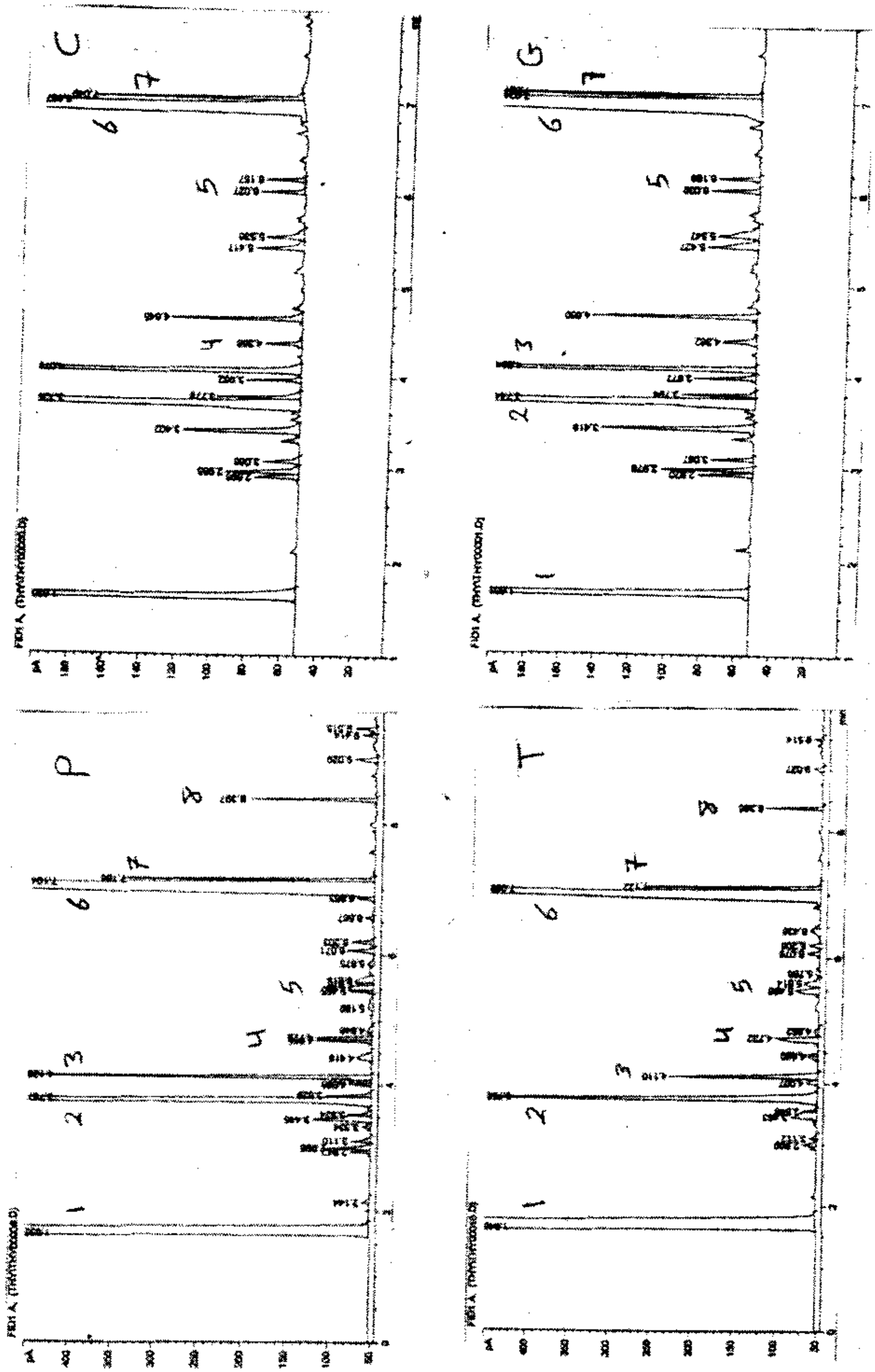

**Fig. (2): Chromatorgam of thyme volatile oil distilled from dried leaves of plants after 6 months of storage.**

**P = Paper bags C = Cotton cloth bags**

**T = Textile polyethylene bags G = Gunny bags.**

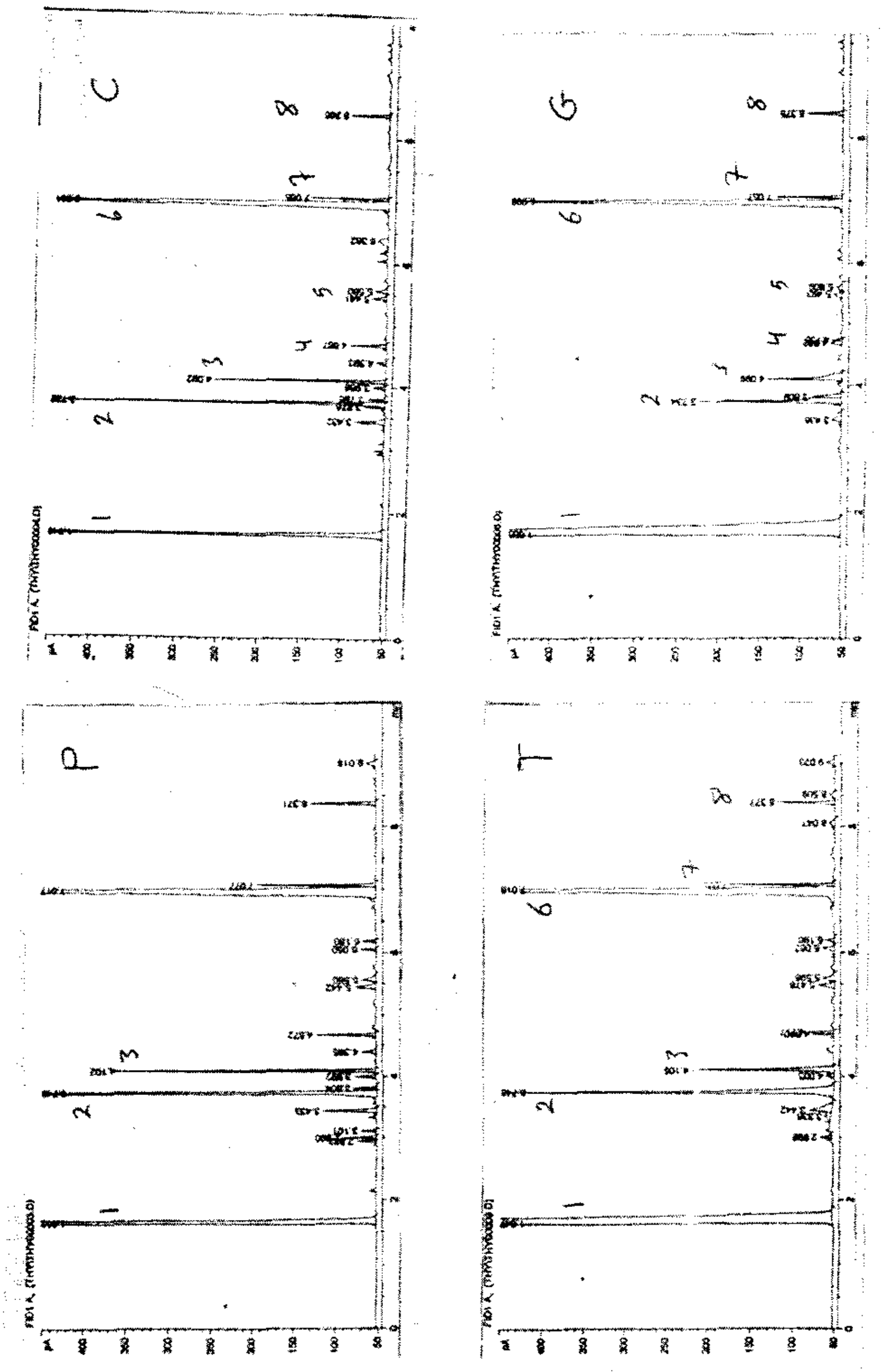


**Fig. (3): Chromatorgam of thyme volatile oil distilled from dried leaves of plants after 12 months of storage.**

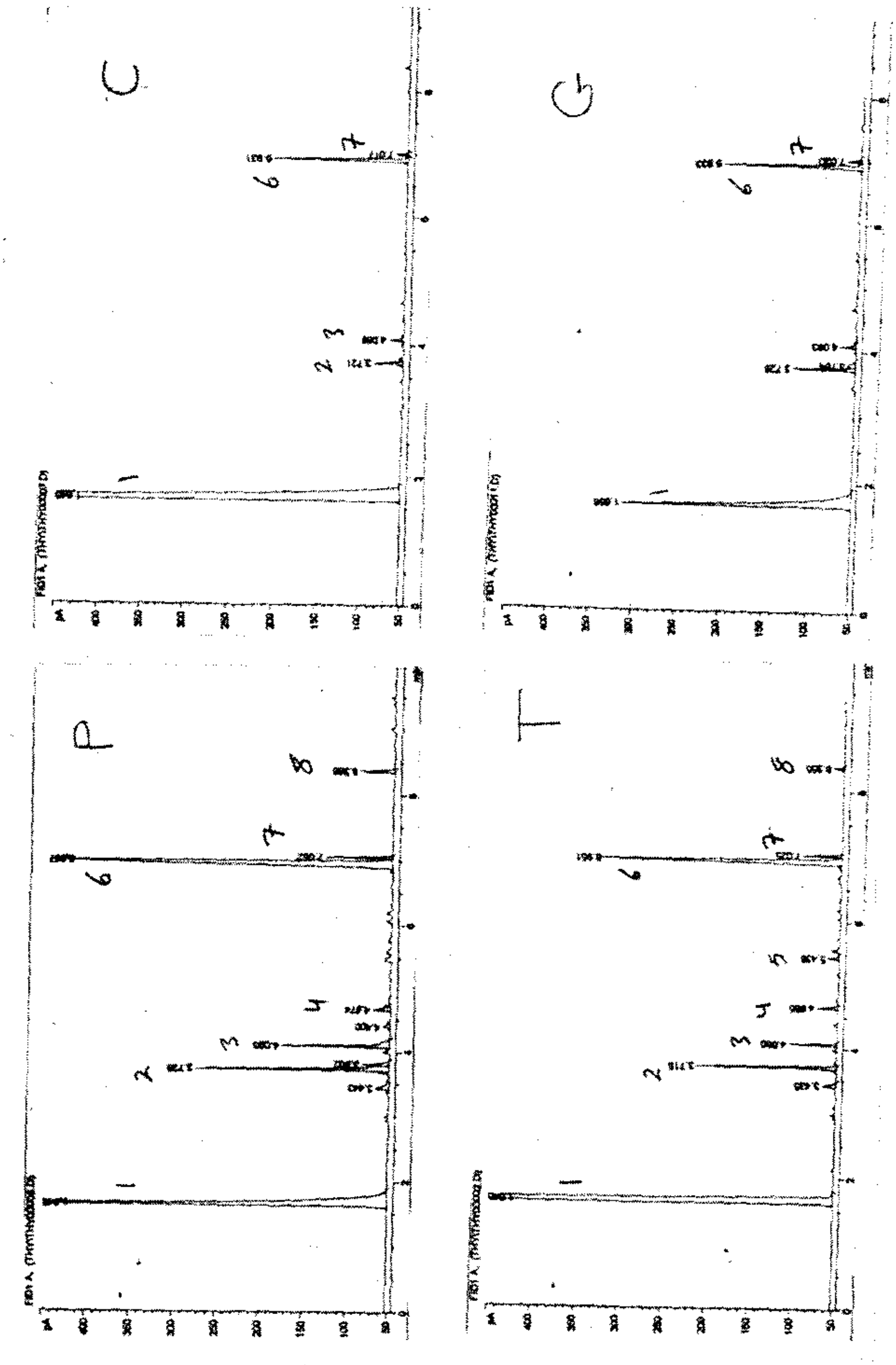

**Fig. (4): Chromatorgam of thyme volatile oil distilled from dried leaves of plants after 18 months of storage.**

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## تأثير مواد التعبئة وفترات التخزين على صبغات الأوراق ومحتوى ومكونات الزيت لنبات الزعتر

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أجرى هذا البحث لدراسة تأثير أربعة مواد تعبئة (أكياس ورقية ـ قماش قطن (كتان) ـ بولى ايثيلين منسوج ذو فتحات ـ أكياس الخيش ذو فتحات) على صبغات الكلوروفيل والكاروتينويدات ونسبة الزيت المستخلص ومكوناته فى الأوراق الجافة المخزنة على درجة حرارة الغرفة لمدد ٣، ٦، ٩، ١٢، ١٨ شهر، ويمكن تلخيص النتائج لمتحصل عليها فيما يلى:ـ

- حدث نقص تدريجى فى المتحوى من صبغات كلورفيل "أ" ، "ب" وكذلك الكاروتين أثناء وبعد التخزين لمدة ١٨ شهرا. هذا وكانت العبوات الورقية أفضل أنواع العبوات فى الحفاظ على نسبة الكلورفيل أ ، ب والكاروتينويدات.
- كان المحتوى من نسبة الزيت الطيار للزعتر ١,٥% فى بداية فترة التخزين وحدث نقص تدريجى حتى نهاية فترة التخزين حيث أصبح ٠,٤٣%، وكان هناك نقص تدريجى معنوى فى نسبة الزيت الطيار خلال فترات التخزين حتى ثمانية عشر شهرا.
- أما من ناحية نوع العبوة وكانت نسبة الزيت مرتفعة فى الأوراق الجافة المخزنة فى العبوات الورقية عن العبوات الأخرى.
- كانت نسبة المركب الفينولى الأساسى (ثيمول) لزيت الزعتر فى بداية فترة التخزين ٤١,٨٥% وقد حدث إنخفاض تدريجى لهذا المحتوى خلال فترة التخزين حتى ١٨ شهرا يصل إلى ١٩,٧٣% فى العبوات الورقية و١٥,٢٥%

فى العبوات المصنوعة من الخيش، بينما يصل إلى ٤,٤٣% و ٧,٦٤% لكل من العبوات المصنوعة من الكتان والبولى إيثيلين المنسوج ويمكن التوصية بتخزين أوراق الزعتر الجافة فى عبوات ورقية ولمدة ١٢ شهر.