

Herb yield and chemical composition of common oregano (*Origanum vulgare* L.) essential oil according to the plant's developmental stage

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Summary

The studies conducted in 2007–2008 were aimed to assess how the developmental stage of common oregano affected the quality and quantity of the herb yield. The field experiment was established with the use of random blocks in four repetitions. Oregano was grown from seeding produced in a greenhouse. Herbage harvest of one-year-old plants was conducted before flowering, in budding phase, at the beginning of flowering and in the full flowering phase. The yield of fresh and air dry herb yield and then the contents of essential oil was determined. The oil was chemically analyzed with the use of GC/MS method. It was demonstrated that the best term for oregano raw material harvest was the full flowering stage of the plants. In this period the yields of fresh and air dry weights as well as the essential oil contents were the greatest. The oil from the herb collected in the initial phase of flowering had the largest number of components, whereas the oil obtained in the budding phase had the smallest. The predominant components of oregano oil were: sabinene, germacrene D, E-caryophyllene, (Z)- β -ocimene and γ -terpinene. The examined oil also contained phenol compounds: thymol and carvacrol – highly bactericidal and fungicidal substances.

Key words: oregano, developmental phases of plant, essential oil, sabinene, germacrene D, E-caryophyllene

INTRODUCTION

Common oregano is applied as a culinary and medicinal herb. It is also highly valued as a decorative and melliferous plant. It blooms from June to the end of vegetation, from August simultaneously produce fruit and seeds. This species is

quite changeable: it creates various, morphologically and chemically differentiated forms, which is related to the place of their occurrence. The *O. vulgare* L. ssp. *hirtum* plants from the Mediterranean climate zone are very rich in essential oil, whereas these originating from the continental climate zone *O. vulgare* L. ssp. *viridulum* and ssp. *vulgare* have less gland hairs and are essential oil –deficient [1-4]. Moreover, the essential oil is characterized by changeable chemical composition, depending on the form of the plant or the place of its distribution [1, 3-9]. Oregano herb is harvested at the beginning of plant flowering: from June to September. In Poland it is mainly obtained from natural state. It is applied in alimentary system diseases and the diseases of upper respiratory tracts. It is an antibacterial, anti-rheumatoid agent, an anti-oxidant and a mild sedative [10-14].

MATERIAL AND METHODS

The field experiment conducted in 2006–2007 was established with the use of random block method in four repetitions. Seeds originated from Planitco Zielonki. The oregano seedling produced in a greenhouse was planted in late May in the distribution of 40 x 40 cm. The surface of one plot was 2.4 m². Herb harvest from one-year-old plants was conducted before flowering, in the 3rd decade of June, in the initial phase of budding, at the end of June, at the beginning of flowering as well as in full bloom, respectively: in mid July and in mid August. Climatic conditions during the vegetation period were shown in table 1. On 10 randomly selected plants in harvest period the height and diameter of the plant, number of shoots and branches on the main shoot, number of flowers and fresh herb weight were determined. The herb yield was also determined on the basis of all plants collected from each plot. After harvest the oregano herb was dried in natural conditions, in a dry, airy and shaded place. Subsequently, the weight of air dried herb was determined and the herb underwent 3-hour distillation in Deryng apparatus. The study results were statistically worked out by means of variance analysis for single classification, with the significance level of $\alpha=0.05$. The analysis of oil chemical composition was conducted using gas chromatography method combined with mass detector, with the use of Varian 4000 GC/MS/MS chromatograph. We applied the VF-5 ms column, 30 m long, with the diameter of 0.25 mm and stationary phase thickness of 0.25 mm. The batcher temperature reached 220°C. We applied 50°C temperature gradient for 1 min, and then increase to 250°C with a speed of 4°C/min and 250°C for 10 min. The carrier gas was helium. Steady flow of 0.5ml/min was applied. One μ l of the solution (1 μ l sample in 1000 μ l of hexane), batcher 250°C, split 1:100 was added. A Varian 4000 MS/MS detector with the registered range 40–1000 m/z, and the scan speed 0.8 sec./scan was used. Kovats' retention indices were determined on the basis of alkane series C6-C40.

Table 1.

Mean air temperature (°C) and total rainfall (mm) for May–August in 2007–2008 and the long term average

| month | air temperature | | | | | | mean air temperature | | 1951–1995 |
|--------|------------------------|------|------------------------|------|------------------------|------|----------------------|------|-----------|
| | 1 st decade | | 2 nd decade | | 3 rd decade | | 2007 | 2008 | |
| | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 | | | |
| May | 9.9 | 11.3 | 15.1 | 13.3 | 19.6 | 13.6 | 14.9 | 12.8 | 13.0 |
| June | 18.2 | 18.0 | 20.0 | 16.4 | 16.2 | 18.8 | 18.1 | 17.7 | 16.4 |
| July | 17.1 | 17.1 | 21.0 | 18.9 | 19.3 | 18.9 | 19.1 | 18.3 | 17.9 |
| August | 17.9 | 19.9 | 18.9 | 20.7 | 18.4 | 17.3 | 18.4 | 19.3 | 17.2 |

| month | total rainfall | | | | | | sum of rainfalls | | 1951–1995 |
|--------|------------------------|------|------------------------|------|------------------------|------|------------------|-------|-----------|
| | 1 st decade | | 2 nd decade | | 3 rd decade | | 2007 | 2008 | |
| | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 | | | |
| May | 13.5 | 57.1 | 29.9 | 34.7 | 37.1 | 9.8 | 80.5 | 101.6 | 57.2 |
| June | 52.4 | 0 | 25.4 | 19.6 | 10.0 | 6.3 | 87.8 | 25.9 | 65.9 |
| July | 48.8 | 39.6 | 35.0 | 19.3 | 3.2 | 18.2 | 87.0 | 77.1 | 73.6 |
| August | 22.3 | 11.4 | 12.9 | 7.0 | 2.4 | 26.6 | 37.6 | 45.0 | 71.1 |

RESULTS

The significant effect of the time of harvesting plants of different developmental phases, on their growth, yield, contents and chemical composition of the essential oil was demonstrated (tab. 2-4). The oregano plants harvested in the vegetative phase, before flowering, had a significantly lowest mean height, number of shoots and branches as well as diameter, as compared to the others. In full flowering period, oregano had significantly greater mean height, number of shots and diameter than other plants. The number of branches in plants harvested in full flowering period was the greatest (12.1 cm) but no significant differences were found between the mean values of plants harvested at the beginning of flowering and in full bloom. The examined oregano plants flowered abundantly: the mean number of flowers on one plant was 142.2 pieces, and in the full flowering period the number of flowers was significantly greater than in the initial phase of flowering.

Table 2.

Influence of harvest time on the growth and development of oregano plants

| term of harvest | plant height [cm] | | | no of shoots per plant | | | no of branches per main shoot | | | plant diameter [cm] | | | no of flowers per plant | | |
|------------------------|----------------------|------|------|---------------------------|------|------|----------------------------------|------|------|------------------------|------|------|----------------------------|-------|-------|
| | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean |
| before flowering | 27.8 | 31.2 | 29.5 | 34.8 | 33.6 | 34.2 | 8.9 | 9.2 | 9.1 | 35.2 | 37.7 | 36.5 | - | - | - |
| budding | 40.3 | 45.9 | 43.1 | 47.6 | 45.7 | 46.7 | 9.2 | 11.4 | 10.3 | 52.6 | 47.5 | 50.1 | - | - | - |
| beginning of flowering | 43.8 | 51.3 | 47.6 | 58.3 | 59.1 | 58.7 | 10.7 | 12.6 | 11.7 | 53.8 | 55.9 | 54.9 | 114.6 | 127.3 | 121.0 |
| full flowering | 56.3 | 62.1 | 59.2 | 71.3 | 68.2 | 69.8 | 11.0 | 13.1 | 12.1 | 56.7 | 61.2 | 59.0 | 168.7 | 159.6 | 164.2 |
| mean | 42.1 | 47.6 | 44.9 | 53.0 | 51.7 | 52.4 | 10.0 | 11.6 | 10.8 | 49.6 | 50.6 | 50.1 | 141.7 | 143.5 | 142.6 |
| LSD _{0.05} | 3.44 | | | 6.21 | | | 1.62 | | | 3.56 | | | 28.32 | | |

Fresh and air dry weight of examined plants was significantly the greatest in the phase of full blooming and equaled, on average, respectively: 175.0 and 55.1g (tab. 3). Thus, the yield of fresh and air dry weight of oregano herb was significantly greater in plants harvested in full bloom, as compared to the others. The yield of fresh oregano herb was on average 0.73kg m⁻², whereas after drying 0.23kg m⁻². No significant differences were found in the yield of plants harvested before flowering and during the budding period. The oregano herb contained on average 0.42% of the essential oil. The concentration of oil was the greatest in the initial phase of flowering (0.58%), however, no significant differences were found in the oil contents of herbs harvested at the beginning of flowering and in full bloom.

Table 3.

Yield and essential oil content of oregano plants in dependence on the term of harvest

| term of harvest | fresh weight of herb [g plant ⁻¹] | | | air dry weight of herb [g plant ⁻¹] | | | yield of fresh herb [kg m ⁻²] | | | yield of air dry herb [kg m ⁻²] | | | oil content [%] | | |
|------------------------|--|-------|-------|--|-------|-------|--|------|------|--|------|------|--------------------|------|------|
| | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean |
| before flowering | 40.9 | 43.7 | 42.3 | 12.0 | 13.5 | 12.8 | 0.17 | 0.18 | 0.18 | 0.05 | 0.06 | 0.06 | 0.21 | 0.19 | 0.20 |
| budding | 95.9 | 89.5 | 92.7 | 28.2 | 24.5 | 26.4 | 0.40 | 0.37 | 0.39 | 0.12 | 0.10 | 0.11 | 0.30 | 0.33 | 0.32 |
| beginning of flowering | 212.7 | 230.1 | 221.4 | 62.1 | 59.6 | 60.9 | 0.89 | 0.96 | 0.93 | 0.26 | 0.25 | 0.26 | 0.53 | 0.62 | 0.58 |
| full flowering | 323.0 | 364.2 | 343.6 | 117.7 | 123.0 | 120.4 | 1.35 | 1.52 | 1.44 | 0.49 | 0.51 | 0.50 | 0.52 | 0.60 | 0.56 |
| mean | 168.1 | 181.9 | 175.0 | 55.0 | 55.2 | 55.1 | 0.70 | 0.76 | 0.73 | 0.23 | 0.23 | 0.23 | 0.39 | 0.44 | 0.42 |
| LSD _{0.05} | 5.34 | | | 8.56 | | | 0.20 | | | 0.04 | | | 0.11 | | |

The chemical composition of oregano oil depended on the developmental stage of plants in herb harvesting period (tab. 4). The predominant components of the oil were sabinene and β -pinene, depending on the year of studies, then β -(Z)- and β -(E)-ocimene, φ -terpinene, E-caryophyllene and germacrene D. Nevertheless, a significant part was played by bicyclogermacrene α -(E,E)-farnesene and, in the second year of studies germacrene-D-4-ol. The contents of thymol and carvacrol

was also found in oregano oil. The presence of the latter compound was reported only in the first year of studies. The amounts of above-mentioned compounds varied with the development of plants. The development of oregano was connected with the increase of sabinene, β -pinene, germacrene D, E-caryophyllene and α -(E,E)-farnesene contents, as well as with the decrease of φ -terpinene, thymol and carvacrol participation.

Table 4.

The main volatiles (%) of oregano plants

| compound | RT | concentration in dependence on the term of harvest | | | | | | | | | | | |
|---------------------------|--------|--|-------|-------|---------|-------|-------|------------------------|-------|-------|----------------|-------|-------|
| | | before flowering | | | budding | | | beginning of flowering | | | full flowering | | |
| | | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean | 2007 | 2008 | mean |
| sabinene | 9.827 | 16.20 | – | – | 15.41 | – | – | 21.67 | – | – | 20.13 | – | – |
| β -pinene | 10.025 | 0.28 | 13.45 | 6.87 | 0.18 | 14.76 | 7.47 | 0.27 | 16.71 | 8.49 | 0.33 | 16.59 | 8.46 |
| β -(Z)-ocimene | 11.918 | 13.22 | 14.36 | 13.79 | 9.12 | 12.01 | 10.57 | 6.99 | 10.69 | 8.84 | 7.95 | 9.90 | 8.93 |
| β -(E)-ocimene | 12.300 | 3.17 | 8.56 | 5.87 | 2.59 | 7.53 | 5.06 | 3.26 | 6.54 | 4.90 | 6.22 | 7.59 | 6.91 |
| φ -terpinene | 12.781 | 10.97 | 2.00 | 6.49 | 6.35 | 1.35 | 3.86 | 2.58 | 1.47 | 2.03 | 2.60 | 1.45 | 2.03 |
| thymol | 21.240 | 2.72 | 0.67 | 1.70 | 1.19 | 0.26 | 0.73 | 0.71 | 0.32 | 0.52 | 0.20 | 0.09 | 0.15 |
| carvacrol | 21.603 | 2.73 | – | – | 0.57 | – | – | – | – | – | – | – | – |
| e-caryophyllene | 25.399 | 6.10 | 11.87 | 8.99 | 12.72 | 10.67 | 11.70 | 11.67 | 9.72 | 10.70 | 10.94 | 8.89 | 9.92 |
| germacrene D | 27.345 | 12.75 | 13.09 | 12.92 | 15.97 | 13.49 | 14.73 | 16.15 | 15.09 | 15.62 | 17.22 | 15.75 | 16.49 |
| bicyclogermacrene | 27.789 | 4.20 | 5.02 | 4.61 | 3.50 | 5.67 | 4.59 | 3.67 | 4.95 | 4.31 | 3.46 | 5.25 | 4.36 |
| α -(E,E)-farnesene | 27.902 | 1.57 | 2.67 | 2.12 | 5.31 | 4.59 | 4.95 | 7.17 | 7.22 | 7.20 | 7.93 | 6.46 | 7.20 |
| germacrene-D-4-ol | 30.250 | – | 6.36 | – | – | 5.97 | – | – | 4.52 | – | – | 5.10 | – |

DISCUSSION

Oregano, as a perennial plant, reaches smaller sizes in the first year of its vegetation than in subsequent years. In this experiment, the plants in their first year of vegetation reached average height of 44.9 cm, diameter of 50.1 cm, and the number of shoots on a plant was on average 52.4; there were also 10.8 branches. These values are in general smaller than those given by Osińska [15], De Mastro et al. [2] and Węglarz et al. [4], but they were close to those obtained by Barreyro et al. [16]. The increased height and diameter of plants, as well as the number of shots, branches and flowers, together with progressive development of plants indicates their harmonious growth and favorable cultivation conditions. The size of plants and oregano herb yield is related to the cultivation conditions, including the amount of water and nitrogen in the soil [16, 17]. Barreyro et al. [16] demonstrated that with the increased dose of nitrogen, up to 80 kg ha⁻¹, the height, diameter and yield of oregano increase. Azizi et al. [17] found out that the deficiency of water in the soil caused the reduction of oregano plant weight but it did not decrease the oil contents. The results of our own studies, as well as those

obtained by other authors, indicate a significant role of cultivation and climatic factors in the formation of oregano yield quality and quantity. In the first year of studies mean fresh plant yield equaled 168.1 g, in second it was 181.9 g. Fresh weight of plants harvested in the full flowering phase in the foregoing experiment was on average 343.6 g and it was close to the mass of certain biotypes analyzed by De Mastro et al. [2]. Osińska [15] demonstrated that oregano herb yield significantly depends on the population and is comprised in the range from 784.0 to 1580 g per plant. Similarly, De Mastro et al. [2] found out that there is a substantial morphological variability of oregano grown in southern Italy.

The amount of essential oil in the oregano herb depends on the population or variation as well as the cultivation conditions. De Mastro et al. [2] report that 1 cm³ of oregano leaf blade may contain from 207 to 734 oil hairs and the amount of essential oil in dry herb is from 0.50 to 5.1%, depending on the population. Studying the morphological-developmental and chemical variability of oregano, Osińska [15] found that the concentration of oil depends on the population and in the climatic conditions of Poland ranges from 0.3 to 1.3%. The oregano plants examined in this experiment contained on average 0.42% of essential oil in air dry herb and from 0.20 to 0.58% , depending on the plant developmental phase. The contents of oil systematically increased with the progressive development of the plant, reaching its maximum in flowering period. Considering the lack of significant differences between mean essential oil contents in oregano herb harvested in the initial phase of flowering and in full bloom, we may say that harvesting plants in these terms makes it possible to obtain high quality yield.

The chemical composition of oregano oil is genetically conditioned and depends on cultivation conditions [1-4, 6-9]. The presented study results reveal that the contents of particular components of oregano oil changes according to the plants' development. The oil from the examined plants harvested in full flowering phase had the greatest contents of sabinene and germacrene D. The contents of sabinene and β -pinene, germacrene-D-4-ol, as well as thymol and carvacrol depended on the climatic conditions and were the most changeable in the study years. Similar relationships were demonstrated by Ivask et al. [6], as they examined the composition of essential oil from oregano grown in Estonia. Lukas et al.[7] report that the main component of Syrian oregano oil is carvacrol and/or thymol simultaneously pointing out to high degree of polymorphism in the presence of these compounds in the particular populations. In the case of the three populations examined by these authors, the predominant compound was carvacrol, and thymol occurred in small amounts, whereas only in one population thymol was the predominant component, and carvacrol occurred in trace amounts. Similar dependences were demonstrated in this paper: when thymol contents in the oil increased, at the same time the concentration of carvacrol decreased, until the absence of this compound in the flowering period. Different results were obtained in Bulgaria by Konakchiev et al. [3], demonstrating that the contents of carvacrol in oregano oil was almost comparable in the budding period and in full bloom.

CONCLUSIONS

1. Growth and yield of oregano herb significantly depended on the plant developmental phase in harvesting period. The best term for herb harvesting, assuring the best yield, was the full flowering phase.
2. It was demonstrated that with the progressive development of oregano plants the essential oil concentration in the herb increases but no significant differences were found in the amount of oil in herbs collected at the beginning of flowering and in full bloom.
3. The chemical composition of oregano essential oil depended upon the plant developmental phase, and its predominant components were: sabinene, β -pinene, β -(Z)- and β -(E)-ocimene, E-caryophyllene, germacrene-D and α -(E,E)-farnesene. The concentration of main compounds differed in particular years of studies.
4. The participation of sabinene, β -pinene, E-caryophyllene, germacrene D and α -(E,E)-farnesene in the oil was increasing, starting from the vegetative, until the generative phase. At that time the contents of thymol and carvacrol were significantly decreasing. The oil from flowering plants did not contain carvacrol.

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PLON ZIELA ORAZ SKŁAD CHEMICZNY OLEJKU ETERYCZNEGO LEBIODKI POSPOLITEJ (*ORIGANUM VULGARE* L.) W ZALEŻNOŚCI OD FAZY ROZWOJOWEJ ROŚLINY

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Streszczenie

Badania przeprowadzone w latach 2007–2008 miały na celu ocenę wpływu fazy rozwojowej lebiodki pospolitej na wielkość i jakość plonu ziela. Doświadczenie polowe założono metodą bloków losowych w czterech powtórzeniach. Lebiodkę uprawiano z rozsady produkowanej w szklarni. Zbiór ziela roślin jednorocznych przeprowadzono przed kwitnieniem, w fazie tworzenia pąków, w fazie początkowego i pełnego kwitnienia. Określono plon świeżej i powietrznie suchej masy ziela, w którym następnie oznaczono zawartość olejku eterycznego. Olejek poddano analizie chemicznej metodą GC/MS. Wykazano, że najlepszym terminem zbioru surowca lebiodki jest faza pełnego kwitnienia roślin. W tym czasie plon świeżej i powietrznie suchej masy był największy, podobnie jak duża była w nim zawartość olejku eterycznego. Olejek pochodzący z ziela zbieranego w początkowej fazie kwitnienia odznaczał się największą liczbą komponentów, najmniejszą natomiast pozyskiwany w fazie tworzenia pąków kwiatowych. Dominującymi składnikami olejku lebiodkowego były: sabinen, germakren D, E-kariofilen, (*Z*)- β -ocimen i γ -terpinen. Badany olejek zawierał również związki fenolowe: tymol i karwakrol, substancje o silnym działaniu bakterio- i grzybobójczym.

Słowa kluczowe: lebiodka, fazy rozwojowe rośliny, olejek eteryczny, sabinen, germakren D, E-kariofilen