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Mendel University in Brno
Faculty of Horticulture Lednice
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Ing. Jiřina Vojtíšková
Ing. Bc. Eliška Peňázová

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2. Vegetable and fruit production, viticulture
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HORTICULTURAL PRODUCTION

VEGETABLE AND FRUIT PRODUCTION, VITICULTURE

GROWTH, FROST TOLERANCE AND ESSENTIAL OIL CONTENT IN DIFFERENT CULTIVARS OF THYMUS VULGARIS L.

Dudaš S.[1] and Böhme M.[2]

[1]Collegium Fluminense Polytechnic of Rijeka, Agricultural Department, Poreč, Croatia
[2]Humboldt-Universität zu Berlin, Dept. Horticultural Plant Systems, Berlin, Germany

Abstract
Thyme (*Thymus vulgaris* L.) is a perennial herb used fresh and dry as spice and as medicinal plant with increasing demand on the market. Because the high economic value of thyme in Europe it was important to study the productivity of seven thyme cultivars in a field experiment in loamy sand for two years. The seedlings were transplanted in the week 17 in plots of a block design with four replicates. Tested cultivars differed in growth velocity, habitus, in the positioning the shoot and branches as well as in the initial flowering. Highest yield of dried leaves was found by the cultivars ´Krajovy´ and ´Deutscher Winter´, highest essential oil content was obtained by hybrid cultivars ´Varico 1´ and ´Varico 2´. Highest frost tolerance showed hybrid ´Varico 1´ and the highest sensitivity was obtained for cv. 'Slonezko'.
Key words: *thyme, cultivations methods, harvesting frequency, frost tolerance, essential oil*

Introduction

Thyme (*Thymus vulgaris* L.) is a perennial herb of the mint family (*Lamiaceae*) used fresh and dry as spice and as medicinal plant with increasing demand on the market. There are information in the literature mostly about chemical composition and essential oil content (Hudaib et al. 2002, Stahl-Biskup and Sáez, 2002, Jordán et al., 2006, Imeloane et al., 2009), aroma profile (Özkan and Chalchat, 2004) chemotype/races of essential oil in thyme found on natural habitat (Stahl-Biskup and Sáez, 2002) furthermore about secretory structures (Svoboda and Svoboda, 2000), medicinal benefits of using thyme, bioactive values and effect of essential oil on human health (Stahl-Biskup and Sáez, 2002; Imeloane et al., 2009). There are some investigations about cultivation methods, including different cultivars (Bundessortenamt, 2002, Vouillamoz et al., 2011), density of plants (Bomme and Nast 1998, Bomme and Wurzinger, 1990, Bomme et al., 1993) and regarding harvesting frequency (Özgüven and Tansi, 1998, Hudaib et al. 2002, Omidbaigi et al. 2005, Golparvar, 2011). In particular information about the cultivar potentials, the harvesting time and frequency are contradictory. Also the influence of fertilisation on the (internal) quality of thyme has been in the focus of scientific research since the beginning of the field production of thyme. After evaluation of the cited literature, it can be concluded that past results regarding the influence of fertilisation on the growth parameters and essential oil content were contradictory (Shalaby and Razin, 1992; Omidbaigi and Arjmandi, 2001; Özgüven et al., 2006). Investigation regarding the influence of different fertilization and plant densities as well as plant protection during the winter confirmed the results of the literature (Böhme and Dudas 2013). Following the literature less information is available concerning the productivity of different thyme cultivars under different cultivations methods and climatic and soil conditions. In this regard the cultivars should be compared also regarding their regeneration after harvesting and the frost tolerance while winter period. In this paper is presented a part of the results of a five year project regarding the productivity of seven cultivars under different growing conditions in different climatic zones of Germany. The focus is on the thyme growth development regarding the flowering period, dried leave yield, essential oil content and the frost tolerance in these seven cultivars.

Materials and Methods

A field experiment with six European (‘Deutscher Winter‘, ‘Varico 1‘, ‘Varico 2‘, ‘Sloneczko‘, ‘De Dolj‘ and ‘Krajovy‘) and one Israeli (‘Typ 8610‘) cultivars of thyme was performed on the experimental field of the Humboldt University of Berlin, in Zepernick. The experimental field can be characterised as follow: 60.2 m above see level; distance to ground water was 150 cm and with a probability of frost for 74-90 days, average year temperature of 8.7°C and the average precipitation 566 mm. The field experiment was set up in a loamy sand (podsol) with a pH 6.4 and organic matter of 1.4 %. In week 17 the thyme plants were transplanted in the field, seven seedlings were planted together on the each planting site to form a shrub. Spacing of the shrubs was 40 cm between the rows and 30 cm between the shrubs in the rows, in six rows with 462 shrubs per plot, in total 12936 shrubs. The experimental plot consisted of four repetitions of 8.75 m² each, whole experiment 307.0 m². Field experiment (design, size of the field experiment, size of the each plot, plant distances and minimale plant number) was created according to Examination Guideline for field trials with MAP from Federal Plant and Variety Office in Hannover (Bundessortenamt, 2004). The nutrient elements in the soil were analysed following the VDLUFA methods. The fertilisation for the thyme plants consisted per ha with 60 kg N, 17 kg P_20_5, 115 kg K_2O, 10 kg MgO and 40 kg CaO, whereas the Nitrogen was applied in three dosages. Harvesting of the herbs was done manually by begin of the flowering. The fresh plant material was dried in a room with 20 to 24 °C and humidity from ca. 50 %. Essential oil content was analysed by water distillation in a Neo Clevenger apparatus. Dried leaves of thyme (30 g) were placed in a 1000 ml flask filled with 400 ml distilled water, the distillation lasted 2 hours. Frost tolerance was evaluated by monitoring of the dead and damaged plants after the winter period for each variety. Collected data were statistically analysed using SPSS software for ANOVA analysis and subsequent tests. Tukey-Test was applied for detection of the significance differences between particular variants/cultivars by a significance level of $P<0.05$.

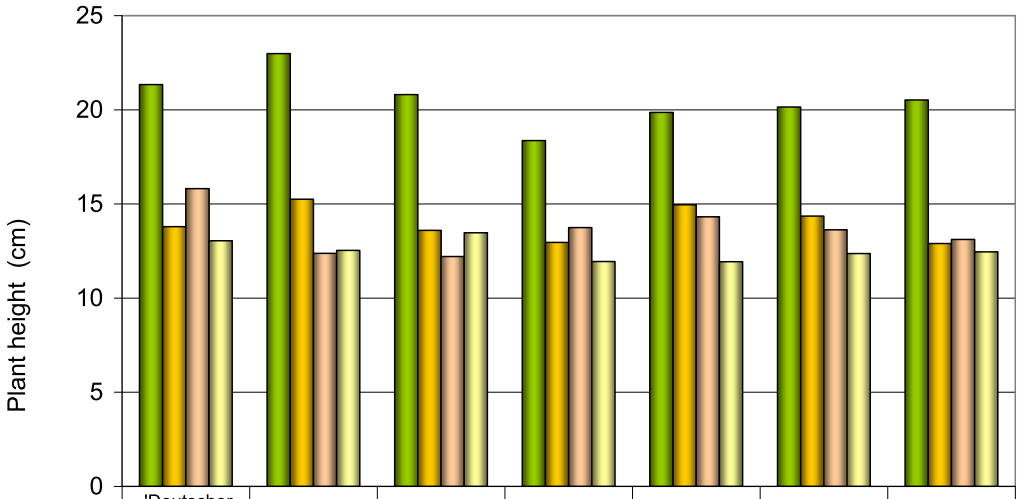
Results and Discussion

According to several authors, there are variable results concerning the optimal harvesting time of herbs, in particular thyme (Özgüven and Tansi, 1998, Hudaib et al. 2002, Omidbaigi et al. 2005, Golparvar, 2011). Most of them are giving the recommendation to start with harvesting at the beginning of flowering, in particular if thyme will be used for spice or tee. If the thyme is cultivated mainly for essential oil yield harvesting is recommended in the fully flowering growth stage. At the stadium of initial flowering, the content of bioactive substances is already high as well as the produced fresh and dry matter. The Table I presents the data about flowering begin for each cultivar during two years of cultivation, short before harvesting was done.

Table I Beginning of the flowering period for the investigated cultivars of thyme

Cultivar	First Year	Second year	Range 1 year	Range 2 year
‘Deutscher Winter‘	14.08.	15.05.	1	3
‘Varico 1‘ H1	-	26.05.	6	7
‘Varico 2‘ H1	-	25.05.	6	6
‘Sloneczko‘	21.09.	20.05.	5	5
‘De Dolj‘	27.08.	11.05.	2	1
‘Krajovy‘	29.08.	17.05.	3	4
‘Typ 8610‘	12.09.	13.05.	4	2

The height of the thyme shrubs by first cut (autumn of the first year) differed between 18.36 and 22.98 cm (figure 1). 'Varico 1' was significant higher than other cultivars. Lowest plant height was found by 'Sloneczko'. Hybrid cultivars 'Varico 1' and 'Varico 2' didn't flowering in the first year of cultivation; regardless, they were harvested in the autumn. Kästner (1966) reported about plant height between 21 and 24 cm in the first year of cultivation. In the second year, plant height varied between 17 and 22 cm before first and 17-18 cm before second harvesting. Better results indicate Hendavy et al. (2010) with variation between 18.75 and 27.25 cm and Azza et al. (2009) with thyme height of 33.7 cm in controlled greenhouse conditions with regular watering, however in warm region of Egypt. Plant height can be a useful indicator in order to compare different cultivars, however for some cultivars, e.g. 'Krajovy' this indicator is not showing the potential of the cultivar. In order to determine the correlation between growth and yield, additional indicator like number of branches or leaf area index (LAI), could give a more representative overview.

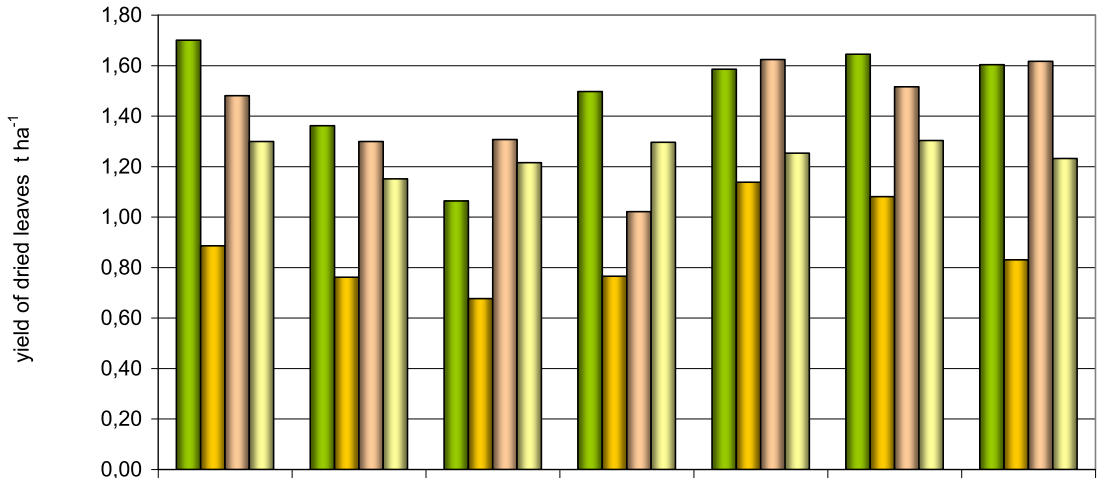


	'Deutscher Winter'	'Varico 1'	'Varico 2'	'Sloneczko'	'De Dolj'	'Krajovy'	'Typ 8610'
1st year 1harvest	21,34 bc	22,98 d	20,8 b	18,36 a	19,86 b	20,14 b	20,52 b
2nd year 1st harvest	13,79 a	15,25 b	13,60 a	12,96 a	14,95 b	14,35 b	12,90 a
2nd year 2nd harvest	15,81 d	12,37 a	12,20 a	13,74 bc	14,31 bc	13,62 b	13,11 b
2nd year 3rd harvest	13,04 ab	12,53 ab	13,47 b	11,94 a	11,93 a	12,36 ab	12,45 ab

Figure 1 Plant heights of thyme before harvesting [cm]. Different letters indicate significant differences in one year according to Tukey ($P<0.05$)

After harvesting, drying process and separating the stems from the leaves, it stays approximately 1/7 -1/8 from the initial fresh matter as yield of dried leaves. The amount of dried leaves per hectare throughout the tested cultivars varied between 1.06 – 1.70 t ha^{-1} for the first cut (figure 2). 'Deutscher Winter' had significantly higher yield of dried leaves than 'Varico 2'. Total yield of dried leaves (cumulative for four harvests) varied between 4.26 tha^{-1} ('Varico 2') and 5.60 tha^{-1} ('De Dolj').

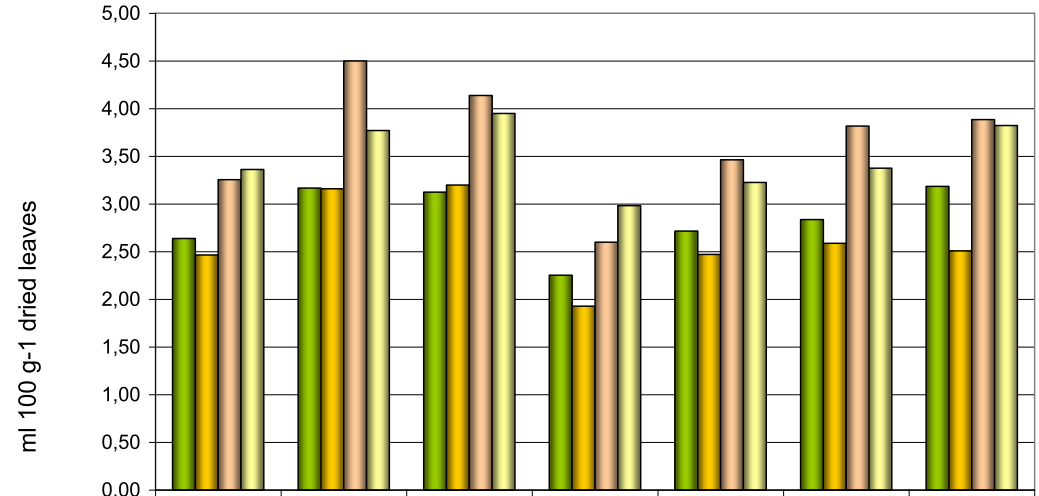
Presented results are in line with information from the literature. Vouillamoz et al. (2011) refers about high variations in total yield of dried leaves throughout various cultivars tested in Swiss. Yield of 'Deutscher Winter' cultivated on the location Malchnau was, in total, 4.31 t ha^{-1} and 5.57 tha^{-1} for in Arbnaz. 'Varico 2' in Malchnau reached yield of dried leaves, in total, of 4.59 t ha^{-1} and in Arbnaz 6.46 tha^{-1}.



	Deutscher Winter	Varico 1	Varico 2	Sloneczko	De Dolj	Krajovy	Typ 8610
1st year 1harvest	1,70 b	1,36 ab	1,06 a	1,50 b	1,59 b	1,64 b	1,60 b
2nd year 1st harvest	0,89 ab	0,76 a	0,68 a	0,77 a	1,14 b	1,08 b	0,83 a
2nd year 2nd harvest	1,48 bc	1,30 b	1,31 b	1,02 a	1,62 c	1,52 bc	1,62 c
2nd year 3rd harvest	1,30 a	1,15 a	1,22 a	1,30 a	1,25 a	1,30 a	1,23 a
cumulative yield	5,37	4,57	4,27	4,59	5,60	5,54	5,28

Figure 2 Yield of dried thyme leaves per harvest and cumulative yield of four harvests [t ha^{-1}]. Different letters indicate significant differences in one year according to Tukey (P<0.05)

The essential oil content in the dried leaves was highest in the hybrids 'Varico 1' and 'Varico 2'. They were significant higher throughout all harvests and compared to all other cultivars (figure 3).



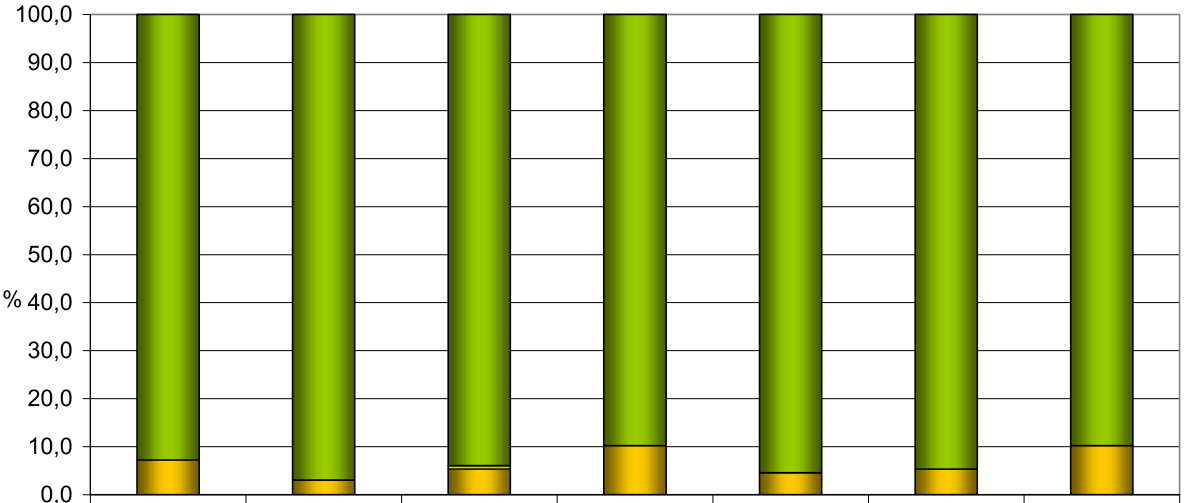
	Deutscher Winter	Varico 1	Varico 2	Sloneczko	De Dolj	Krajovy	Typ 8610
1st year 1harvest	2,64 b	3,17 c	3,13 c	2,25 a	2,72 b	2,84 b	3,19 c
2nd year 1st harvest	2,47 b	3,16 c	3,20 c	1,93 a	2,47 b	2,59 b	2,51 b
2nd year 2nd harvest	3,26 b	4,50 e	4,14 d	2,60 a	3,46 b	3,82 c	3,89 c
2nd year 3rd harvest	3,36 b	3,77 c	3,95 c	2,98 a	3,23 b	3,38 b	3,82 c

Figure 3 Essential oil content in thyme cultivars [ml 100 g^{-1} dried leaves]. Different letters indicate significant differences in one year according to Tukey (P<0.05)

In the summer period of the second year of thyme cultivation, both 'Varico' hybrids started with fully flowering, this resulted in increasing content of essential oil from harvest to harvest. Even in the last harvest, in autumn of the second year, hybrids gave drug (dried

leaves) with significantly highest essential oil content. High essential oil content in hybrid cultivars 'Varico 2' were also stated in investigations of Vouillamoz et al. (2011). Essential oil content by cultivar 'Varico 2' in Switzerland was 2.9-3.4 % and by 'Deutscher Winter' with 2.4-2.9 % cultivated on two different locations. Earlier investigation showed essential oil contend for 'Deutscher Winter' in the first harvest between 1.82 and 2.17 % as well as 2.35 and 2.61 % for the second cultivations year (Kästner, 1966).

After first winter the loss of the plant were monitored: healthy, destroyed and part injured plants were detected. The frost tolerance of all cultivars was satisfying; the amount of part destroyed and injured plants varied between 3.03 to 10.23 % by the cultivars (figure 4). Highest frost tolerance showed hybrid ´Varico 1´.



	Deutscher Winter	Varico 1	Varico 2	Sloneczko	De Dolj	Krajovy	Typ 8610
HP	92,8	96,97	93,95	89,77	95,45	94,7	89,77
DP	0,00	0,00	0,75	0,00	0,00	0,00	0,00
PDP	7,20	3,03	5,30	10,23	4,55	5,30	10,23

HP- healthy plants/shrubs DP- dead, destroyed plants/shrubs PDP- partially dry-injured plants/shrubs

Figure 4 Frost tolerance of the thyme cultivars after first winter period [%]

Conclusions

The plant height can be a useful indicator for evaluating the vitality of thyme. On the other hand more precisely statements are possible if these data correlate to the plant fresh and dry mass.

The amount of essential oil content corresponded in all thyme cultivars the international standard regulation.

Following the breeding strategy in the past, older cultivars were selected in order to have high biomass production. In this regard highest yield of dried leaves were obtained with traditional cultivars ´Krajovy´ and ´Deutscher Winter´.

Hybrid cultivars ´Varico 1´ and ´Varico 2´ showed a very homogenous growth, but characterised in slower plant growth than other cultivars.

Obviously hybrids have the highest potential in productivity, the highest content of essential oil in dried leaves was detected by hybrid cultivars ´Varico 1´ and ´Varico 2´.

It seem s all cultivars are appropriate for middle Europe climate conditions, because hibernation status was satisfying. The amount of partly destroyed and injured plants varied by the cultivars between 3.03 to 10.23 %.

Summary
The aim of this paper was to present a part of the results of a five year project regarding the productivity of seven cultivars under different growing conditions in different climatic zones of Germany. The focus is on the thyme growth development regarding the flowering period, dried leave yield, essential oil content and the hibernation in these seven cultivars. A field experiment with six European and one Israeli cultivars of thyme was performed on the experimental field of the Humboldt University of Berlin Evaluation of the indicators for productivity as mentioned was conducted for the cultivars: ´Deutscher Winter´, ´Varico 1´, ´Varico 2´, ´De Dolj´, ´Krajovy´, ´Sloneczko´ and ´Type 8610´. Monitored were hibernation, share of destroyed plants, beginning of the flowering, yield capacity (fresh herb and dried leaves) and qualitative potential in essential oil content. Collected data were statistically analysed using SPSS software for ANOVA and supporting tests to find the significant differences between cultivars (Tukey test) by significance level $P<0.05$.

Literature/References
AZZA, A. EZZ EL-DIN, A., AZIZ, E. E., HENDAWY, S.F., OMER E.A., 2009: Response of *Thymus vulgaris* L. to Salt Stress and Alar (B) in Newly Reclaimed Soil. Journal of Applied Sciences Research, 5, 12: 2165-2170. ISSN 1816157X
BÖHME, M., DUDAŠ, S. 2013: Influence of Nitrogen and Potassium fertilisation and cultivation methods on the yield and essential oil content in thyme (*Thymus vulgaris* L.) NUTRIHORT: Nutrient management, nutrient legislation and innovative techniques in intensive horticulture, September 16-18, 2013, Ghent, Belgium, 58: 45, ISBN: 978-9040303463
BOMME, U., NAST, D. 1998: Nährstoffentzug und ordnungsgemäße Düngung im Feldanbau von Heil- und Gewürzpflanzen. Zeitschrift für Arznei- & Gewürzpflanzen, 3: 82-90, ISSN 1431-9292
BOMME, U., NAST, D., RINDER, R., VOIT, K. 1993: Untersuchungen über Nährstoffentzug und umweltgerechte Düngung von Heil- und Gewürzpflanzen im feldmäßigen Anbau. Gartenbauwissenschaft, 58, 1: 25-31, ISSN 0016-478X
BOMME, U., WURZINGER, A. 1990: Nmin-Bodenuntersuchungsergebnisse im Heil und Gewürzpflanzenbau. Gemüse, 3: 176-178, ISSN 0016-6286
BUNDESSORTENAMT 2004: Richtlinien für die Wertprüfung mit Heil- und Gewürzpflanzen. Richtlinie für die Wertprüfungen von Thymus vulgaris L., 59-62. ISBN -
BUNDESSORTENAMT, 2002: Beschreibende Sortenliste Arznei- und Gewürzplanzen. Deutscher Landwirtschaftsverlag GmbH, Hannover, 164-169, ISBN 1617-4569
GOLPARVAR, A. R., 2011: Determination of the Best Harvesting Times to Obtain Maximum Dry Herbage, Essential Oil and Thymol Yield in Garden Thyme (*Thymus vulgaris* L.). International Journal of Life Science and Medical Research, 1: 1-4, ISSN 2226-4558
HENDAWY, S.F., EZZ EL-DIN, A., AZIZ, E. E., OMER E.A. 2010: Productivity and oil quality of *Thymus vulgaris* L. under organic fertilization conditions. Ozean Journal of Applied Sciences 3, 2: 203-216, ISSN 1943-2429
HUDAIB, M., SPERONI, F. and CAVRINI, V., 2002: GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. Journal of Pharmaceutical and Biomedical Analysis, 29, 4: 691-700, ISSN 0731-7085
IMELOANE, B., AHMAMDI; H., WATHELET; J.P., ANKIT, M., KHEDID, K., EL BACHIRI, A., 2009: Chemical Composition and Antimicrobial Activity of Essential Oil in Thyme (*Thymus vulgaris*) from Eastern Marocco. International Journal of Agriculture & Biology, 11, 2: 205-208, ISSN 1814-9596

JUÁREZ, R. C. R., CRAKER, L. E., MENDOZA, N. R. AGUILAR-CASTILLO, J. A., 2011: Humic substances and moisture content in the production of biomass and biactive constituents of *Thymus vulgaris* L. Rev. Fitotec. Mex., 34, 3: 183 – 188, ISSN: 0187-7380

JORDÁN, M. J., MARTINEZ, R. M., GOODNER, K. L., BALDWIN, E.A., SOTOMAYOR, J. A., 2006: Seasonal variation of Thymus hyemalis Lange and Spanish *Thymus vulgaris* L. essential oils composition. Industrial Crops and Products, 24, 3: 253–263, ISSN: 0926-6690

KÄSTNER, G. 1966: Untersuchung über den Einfluß der Aussaat- und Erntezeit sowie der Schnitthöhe auf den Ertrag und die Qualität des Thymians – *Thymus vulgaris* L., Inauguraldissertation an der Landwirtschaftlichen Fakultät der Karl –Marx Universität Leipzig, 208 p.

OMIDBAIGI, R., ARJMANDI, A. 2001: Effects of NP-Supply of Growth, Development, Yield and Active Substances of Garden Thyme (*Thymus vulgaris* L.). Proceedings of the World Conference of Medicinal and Aromatic Plants „Possibilities and Limitations of Medicinal and Aromatic Plants Production Towards the 21st Century“, July, 08.-10., Budapest, Hungary /Acta Horticulturae 2002, 576, 263-265, ISBN 978-90-66058-75-0, ISSN 0567-7572

OMIDBAIGI, R., SEFIDKON, F., HEJAZI, M., 2005: Essential oil composition of *Thymus x citirodorus* L. cultivated in Iran. Flavour and Fragrance Journal, 20: 227-238, ISSN 1099-1026

ÖZGÜVEN, M. and TANSI, S., 1998: Drug Yield and Essential Oil of *Thymus vulgaris* L. as in Influenced by Ecological and Ontogenetical Variation. Turkish Journal of Agriculture and Forestry, 22: 537-542, ISSN 1300-011X

ÖZGÜVEN, M., AYANOG, F. and A. OZEL, 2006: Effects of Nitrogen Rates and Cutting Times on the Essential Oil Yield and Components of Origanum syriacum L. var. bevanii. Journal of Agronomy 5 (1): 1 01-105,

ÖZKAN, M. and CHALCHAT, J.C., 2004: Aroma profile of *Thymus vulgaris* L. growing wild in Turkey. Bulg. J. Plant Physiol. 30, 3-4: 68-73, ISBN 1310 4586

SHALABY, A. S. and RAZIN, A. M. 1992: Dense Cultivation and Fertilisation for Higher Yield of Thyme (Thymus vulgaris L.). Journal of Agronomy & Crop Science, 168: 243-248

STAHL-BISKUP, E. and SÁEZ, F., 2002: *Thyme. The genus Thymus.* Medicinal and Aromatic Plants - Industrial Profiles, Tailor and Francis, London and New York, ISBN 041 5-28488-0

SVOBODA, K. AND SVOBODA, T. G., 2000: Secretory Structures of Aromatic and Medicinal Plants. Microscopix Publications, Middle Travelly, Beguildy, Knighton. Powys U.K, ISBN 0-953846 1-0-5

VOUILLAMOZ, J. F., SCHALLER, M., ROSSINELLI, M., CARRON C.–A. and CARLEN, C., 2011: 'Varico 3', nouvel hybride de thym (*Thymus vulgaris* L.) pour la production en Suisse. Revue suisse Viticulture, Arboriculture, Horticulture, 43, 6: 370–376, ISSN 0375-1430

Address

Dr. Slavica Dudaš, Collegium Fluminense Polytechnic of Rijeka, Agricultural Department Poreč, Carla Huguesa 5, Poreč, Croatia, sdudas@veleri.hr

Prof. Dr. sc. Dr. h.c. mult Michael Böhme, Humboldt Universität zu Berlin, Faculty of Agriculture and Horticulture, Dept. Horticultural Plant Systems, Lentzeallee 75, Berlin, Germany, michael.boehme@cms.hu-berlin.de