Carpathian Journal of Earth and Environmental Sciences, February 2013, Vol. 8. No. 1, 217 - 222

DATA REGARDING THE ESSENTIAL OILS FROM SEVEN POPULATIONS OF *THYMUS BALCANUS* BORBÁS

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Abstract: Numerous essential oils from plants belonging to the family *Lamiaceae* and commonly used in food industries, cosmetics, aromatherapy, etc. have shown to have significant pesticide properties. Species belonging to the genus *Thymus* show a number of variations in the chemical composition of secondary products. These variations represent an opportunity to study the ecological role of these essential oils. In this paper the authors investigate the chemical composition of essential oils from seven populations of *Thymus balcanus*, in order to identify natural populations with a high pesticide potential. The vegetal material was collected during flowering period from the Făgăraş Mountains, from different altitudes (1100-2000 m). The essential oil has been extracted using a NeoClevenger apparatus and the separation and the identification of the components have been carried out using a GC-MS Agilant 6890 N with a spectrometric mass detector 5973 and an auto sampler. Significant essential oil polymorphism was found in the chemical composition of volatile oils of *Thymus balcanus*, probably representing a way of adapting to different habitat conditions. Thus, at hight altitude the main constituent of essential oil was found to be thymol (23.60%), followed by linalool (17.63%) and linalool acetate (16.12%) and at low altitude the main constituent is farnesol, followed by germacrene D, β-caryophyllene, thymol, carvacrol, estragol and linalool.

Key words: Thymus balcanus, natural pesticide, essential oils, Făgăraş Mountains, environmental impact

1. INTRODUCTION

The genus *Thymus* is part of the *Lamiaceae* family and is considered to be one of the most important genera of this family because of the large number of species from which it is formed (Morales, 2002). At this moment there are still challenges regarding the taxonomy of species belonging to this genus due to high populational variability, even if there are data on morphology, micromorphology, chemical composition and products resulting from secondary metabolism (Dajic & Śoštarić, 2006). The essential oils of *Thymus* species present a high chemical variability due to different intrinsic (genetic variation) and extrinsic (ecological and environmental aspects) factors (Stahl-Biskup, 2002). Environmental factors such as temperature, radiation and photoperiod play a significant role in the quantity and quality of the essential oil (Yamaura et al., 1989; Pluhar et al., 2007). Also, the nutrient material that is vital to plant growth, such as water, mineral elements and nitrogen, play an important role in the chemical composition and quality of the essential oil (Rajeswara et al., 1990).

Essential oils are particularly important in plant life, although they have long been considered waste as a result of plant metabolism (Stahl-Biskup, 2002). Their importance derives from the fact that they are a true chemical barrier against the attack of pests, pathogens of ruminants, being involved in plant protection against some environmental factors.

On the other hand they greatly reduce water loss from the leaves. Also seems to play an important role in pollination by attracting pollinating insects. All these activities could be considered in developing pest management strategies which include better environmental protection (Regnault-Roger & Hamraoui, 1997).

Species belonging to the genus *Thymus* (rich in monoterpenes, thymol and carvacrol in particular), can be a source of plant pesticides. Thus, volatile oils of *Thymus* sp. were used against *Anathoscelides obtectus* Say., frequent pest that damages its host plant, the kidney bean (*Phaseolus vulgaris* L.) in the field and during storage (Regnault-Roger et al., 1993).

The essential oils of *Thymus vulgaris* show activity against *Tetranychus urticae,* an agricultural pest with an extensive host plant range and an extreme record of pesticide resistance (El-Gengaihi et al., 1996). Lee et al., (1997) analyzed the toxicity of 34 natural monoterpens against three important arthropod pest species: the larvae of the Western corn rootworm (*Diabrotica virgifera* LeConte), the adult two-spotted spider mite (*Tetranychus urticae* Koch*)* and the adult house fly (*Musca domestica* L.). Their research showed that thymol was the most toxic terpene against house fly, and citronellol and thujone were the most effective on the Western corn rootworm.

In this paper the authors investigate the essential oils from seven populations of *Thymus balcanus,* populations that were taken from areas with different altitudes. Our goal was to identify natural populations of *Thymus balcanus* with a high potential pesticide, knowing that natural products are an excellent alternative to synthetic pesticides, reducing the negative impact on human health and the environment.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *Thymus balcanus* were collected during flowering period from Făgăraş Mountains. *Thymus balcanus* Borbás is a synonym for *Thymus praecox* Opiz subsp. *polytrichus* (A. Kern ex Borbás) Jalas (Jalas, 1972). This species grows in alpine and subalpine regions especially on crystalline rocks. In Romania, it is frequently found in all mountain areas. The plant presents long, somewhat woody, creeping branches, non-flowering or with a terminal inflorescence. Leaves are mostly obovate, broadly spathulate to suborbicular, ciliate at the base, with lateral veins prominent and marginal veins present in the upper part (Jalas, 1972).

The vegetal material was harvested from areas at different altitudes (1100m – 2000m) and was identified by dr. Ioan Sarbu (Batanical Garden, Iasi, Romania).

2.2. Isolation of essential oils

Aerial parts of plant material were subjected to hydrodistillation, using a NeoClevenger apparatus, according to the method recommended by the European Pharmacopea (Maissoneuve, 1983). The obtained essential oils were stored at +4^{o}C until analysis.

2.3. Analysis of essential oils

The separation and the identification of the components of essential oils have been carried out using GC-MS (gas chromatography coupled with mass spectrometry) Agilant 6890 N with a spectrometric mass detector 5973 and an auto sampler. The DB5 chromatographic column has a length of 30 m an interior diameter of 0.25mm and a film diameter of 0.25μm. The separated compounds were identified by means of the Nist spectrum database, and the peak position was confirmed by the Kovats retention index.

3. RESULTS AND DISCUSSIONS

3.1. Chemical composition of essential oils

The use of synthetic pesticides causes numerous problems for the environment and for humans, due to their high toxicity. The residues from soil, water and crops can affect public health (Koul et al., 2008). For these considerations natural pesticides become impetuous required. They are more compatible with the environmental components than synthetic pesticides (Isman & Machial, 2006).

It has been shown that volatile oils, besides their many properties (antimicrobial, antioxidant, anthelmintic, etc.) can be successfully used as natural pesticides, with double effect on insects: either directly on adult insect or inhibiting reproduction (Regnault-Roger & Hamraoui, 1997). The most effective essential oils from this point of view are those from the *Lamiaceae* and *Myrtaceae* family (Isman, 2000). Regarding the localization in plants of essential oils, these are found in glandular hairs, being present in leaves, flowers and stems (Bruni & Modenesi, 1983).

A high chemical variability was observed in the essential oils of the various species of the *Thymus* genus, according to ontogenetic stage of the plants and environmental conditions. Only the essential oils that contain high amounts of phenols (thymol and carvacrol) were found to be important as natural pesticides (Regnault-Roger et al., 1993).

Concerning the essential oils of *Thymus balcanus*, in a survey of available literature, only few publications relevant for the chemical composition were found, suggesting that a substantial lack of information exists in this field

(Kisgyörgy et al., 1983; Kulevanova et al., 1998). Previously reported results on volatile oil composition of *Thymus balcanus* (Kulevanova et al., 1998) indicated that the major components were α-terpineol, linalool, linalyl acetate, α-terpinyl acetate and neryl acetate. Regarding the pesticide potential of essential oils of *Thymus balcanus*, from the available literature, no studies were identified for this purpose. Our study came to complete these goals, both in terms of chemical composition of essential oil of *Thymus balcanus* and also, its potential use as a natural pesticide.

Analyzing the chemical composition of the essential oil extracted from *Thymus balcanus*, collected from different altitudes, a total of 66 substances have been identified, which represent 91.15% - 98.78% of the total substances detected on the column (Table 1).

Thus, at 2000 meters, a total of 36 compounds have been identified, the share is owned by thymol (23.60%), linalool (17.63%) and linalool acetate (16.12%). At 1980 meters, a number 34 of substances have been identified, the most important being thymol (33.85%). Also, thymol has proved to be the most important compound of the volatile oil in *Thymus balcanus* populations collected from 1790 meters, at this altitude a number of 41 compounds being identified. At 1690 meters, 32 substances have been identified, the main ones being thymol (35.41%) and linalool (19.98%).

In the essential oil obtained from *Thymus balcanus* populations collected at the altitude of 1585 meters we have identified a number of 32 compounds, in the largest percentages finding thymol ((30.58%) and carvacrol (24.15%).

At 1400 and 1100 meters, 43 chemical compounds have been identified, farnesol (20.07%) and thymol (16.85%) being the main constituents in the biological material growing at 1.400 meters, while farnesol (33.51%), germacren D (16.58%) and β-caryophyllene (13.42%) have been found as preponderant in plants collected at the altitude of 1100 meters.

The volatile oil extracted from plants collected from higher altitudes recorded an increased level of phenolic monoterpenes, of which thymol had the largest proportion (35.41% at 1690 meters, and 23.60% at 2000 meters). These results confirm the results obtained by Kokkini (1997) in *Origanum vulgare*, whereby plants collected in the mountainous area of Greece have a higher content in thymol (18.8–26.6%), while the original plants from lower altitudes had a higher content of carvacrol (5-7.4%).

Table 1. The composition of essential oils extracted from *Thymus balcanus* plants collected from different altitudes

No.	Compound	Altitude						
		2000m	**1980m**	**1790m**	**1690m**	**1585m**	**1400m**	**1100m**
		Percentage						
1.	α-Thujene	-	-	-	-	0.25	-	0.07
2.	α-Pinene	-	-	-	-	0.11	0.68	1.45
3.	Camphene	-	-	-	-	-	1.27	2.63
4.	β-Pinene	-	-	-	-	-	-	0.31
5.	Sabinene	-	-	-	-	-	0.16	-
6.	Octen-3-ol	0.58	0.65	0.60	0.51	0.62	0.19	0.13
7.	β-Octenon	-	-	0.14	-	-	-	-
8.	β-Mircene	0.27	0.26	0.36	-	0.42	0.16	0.10
9.	Limonene	-	-	-	-	-	-	0.92
10.	3-Octanol	-	0.19	0.21	0.17	0.33	-	-
11.	α- Terpinene	-	-	-	-	0.39	0.11	-
12.	o-Cimene	0.97	1.62	1.59	0.92	2.4	1.34	-
13.	Eucalyptol	0.58	0.31	0.50	0.97	0.35	0.68	-
14.	trans- β-Ocimene	-	-	0.09	-	0.16	0.11	0.12
15.	γ-Terpinene	0.47	0.98	1.04	0.41	3.70	0.70	0.07
16.	Isopropyl methyl biciclo hexan-2-ol	1.99	2.52	4.46	0.21	0.09	0.12	0.26
17.	Linalool	**17.63**	6.85	9.37	**19.98**	0.33	9.42	2.14
18.	Camphor	-	-	-	0.51	4.47	0.28	0.14
19.	Borneol	-	-	-	-	-	0.23	0.18
20.	Octenyl acetate	0.24	0.13	0.18	-	-	-	-
21.	Menthone	0.36	0.19	0.31	0.24	4.98	-	-
22.	Borneol	0.57	0.52	0.69	-	1.45	-	-
23.	Terpinen-4-ol	0.24	0.22	0.45	-	0.17	0.19	0.30

24.	α-Terpineol	2.35	3.83	2.05	1.32	-	0.43	0.11
25.	Estragol	0.47	2.35	4.46	9.72	-	7.01	2.03
26.	Nerol	0.57	-	0.23	-	-	-	-
27.	Metil timil eter	0.91	1.83	1.20	0.39	1.04	0.35	0.15
28.	cis-Geraniol	-	-	-	-	-	0.16	-
29.	Thymoquinone	-	-	-	-	0.18	-	-
30.	Linalool acetate	**16.12**	8.36	7.86	2.10	-	1.45	0.40
31.	Thymol	**23.60**	**33.85**	**31.17**	**35.41**	**30.58**	**16.85**	5.44
32.	Methyl acetate	-	-	-	-	1.08	-	0.34
33.	Carvacrol	5.89	4.90	5.56	5.42	**24.15**	2.23	0.59
34.	Menthadienol	0.94	0.56	1.28	-	-	-	-
35.	α-Terpinyl acetate	0.64	-	-	-	-	-	-
36.	Timil acetate	0.39	0.16	0.77	0.63	0.77	0.47	0.21
37.	Neryl acetate	0.13	0.55	0.17	-	0.58	-	-
38.	β-Burbonene	-	-	-	-	0.09	0.27	0.70
39.	Geranyl acetate	5.22	6.10	2.53	0.68	-	0.76	-
40.	β-Elemene	-	-	-	0.25	-	1.30	0.43
41.	β-Caryophyllene	6.07	8.55	7.42	7.23	6.26	5.93	**13.42**
42.	α-Farnesene	-	-	-	0.21	-	-	-
43.	α-Bergamoten	-	0.23	0.24	-	-	0.64	0.18
44.	α-Guaien	-	-	-	-	-	-	0.23
45.	α-Caryophyllene	0.33	0.43	0.51	0.46	0.28	0.81	0.83
46.	Biciclosesquifelandren	-	-	-	-	-	0.66	-
47.	Alloalomadendren	0.15	-	0.32	-	-	-	-
48.	Germacrene D	1.05	1.42	1.62	2.82	2.55	7.27	**16.58**
49.	Elixene	0.59	1.00	0.93	0.83	0.41	1.12	0.73
50.	α-Farnesene	-	-	-	-	-	-	1.13
51.	β-Bisabolene	0.69	1.46	0.89	1.89	1.17	1.52	0.48
52.	γ-Cadinene	0.28	0.37	0.45	0.57	-	1.66	0.27
53.	β- Cadinene	-	-	0.17	-	-	-	-
54.	cis- α-Bisabolene	-	0.50	0.70	0.18	0.38	-	-
55.	Nerolidol	-	-	0.24	0.93	-	-	0.23
56.	Geranyl izobutyrate	0.10	-	-	-	-	-	-
57.	γ-Neurolene	1.83	2.18	3.08	-	0.64	1.21	0.75
58.	Cariophyllene oxide	0.82	1.43	0.94	0. 51	0.77	1.00	1.42
59.	Cubenol	0.15	-	0.20	0.25	-	1.15	0.25
60.	tau-Cadinole	1.75	1.74	1.60	2.18	-	5.52	2.26
61.	α-Cadinal	-	0.25	-	-	-	-	-
62.	tau-Neurolol	0.46	-	0.35	0.21	-	0.91	0.88
63.	Lead oxide	-	-	-	-	-	0.66	1.51
64.	Farnesal	-	-	-	-	-	0.52	1.06
65.	Farnesol	-	-	-	1.18	-	**20.07**	**33.51**
66.	trans-Farnesol	-	-	-	-	-	0.70	1.55
	Total %	**95.40**	**96.49**	**96.93**	**98.78**	**91.15**	**98.27**	**96.49**

3.2. Environmental impact

It is important to analyze the variations that occur in the chemical composition of essential oils to better understand their ecological significance. A more detailed analysis of the studied essential oils shows that on the level of monoterpenes (volatile compounds formed from two isoprene units and the first separated in the chromatographic column) there are a number of qualitative and quantitative differences, depending on the area in which the biological material was collected.

Many studies (Gouyon et al., 1987; Granger et al., 1963; Vernet et al., 1977) highlight that the variation of monoterpenes could be an adaptive strategy in relation to environmental variations.

The ecological aspects of the role of essential oils are reflected in the interaction of plants with environmental factors, so the natural selection favors survival of the population with the composition of essential oil with a higher adaptive value (Stević et al., 2011).

An important issue is the environmental pollution with pesticides, problem who created serious risks not only regarding the disappearance of some species (mainly plant species), but also on human health damage due to contamination of groundwater. Some agricultural products, which contain small amounts of pesticides, can exert long-term adverse effects on human health but also on animals who consume these products (Corbu & Cachita-Cosma, 2010).

It is estimated that approximately 2.5 million tons of pesticides are used annually in crops and the damage caused by these pesticides amounts over 100 billion dollars annually. The high level of the toxicity of these pesticides is because of its non-biodegradable properties, affecting the soil, the water resources and crops, in this way having a negative impact on human health (Koul, 2008).

To prevent or eliminate such shortcomings Narwal (1996) recommended the application of pesticides with more discernment, or even their total elimination of agro-procedures and replacing them with alternative strategies based on allelocompounds. The allelopathy, chemical interaction between plants, including stimulatory and inhibitory influences (Molisch, 1937), play an important role in nature. Suitable manipulation of the allelopathy towards the improvement of crop productivity and environmental protection through natural products has gained a special attention of scientists engaged in allelopathy researches (Safari et al., 2010).

From this point of view the essential oils from populations containing large amounts of thymol may be successfully used as pesticides. A number of studies have shown that monoterpenes, especially thymol, rapidly degrade in the environment presenting low risk to the environment, offering a safe alternative to other more persistent chemical pesticides that degrade more slowly and can cause contamination of the soil (Hu & Coats, 2008). So, we can say that populations of *Thymus balcanus* that grow at higher altitudes and that present large quantities of thymol in essential oils composition may be successfully used as natural sources of pesticides.

Pesticides derived from essential oil of plants present several important benefits. Because the natural pesticides contain volatile substances the level of risk to the environment and human is reduced. Also, due to the large number of compounds that form volatile oils, the resistance of these oils will develop more slowly (Opender et al., 2008).

4. CONCLUSIONS

The studies of *Thymus* sp. secondary metabolic products, especially the essential oils, present a particular importance because of the ecological role of its compounds. It is known that essential oils have a role in plant adaptation to abiotic environment, achieving competitive interactions with other herbs (alelopathy) and also serve as defense against herbivores and pathogens. Most likely biotic and abiotic environmental factors do not act individual, but together, leading to a chemical polymorphism of the essential oils.

Knowing that natural products represent a very good alternative to synthetic pesticides is important to identify natural population of plants that produce essential oil with a high potential pesticide. Analyzing essential oils from seven populations of *Thymus balcanus*, we found that populations that grow at higher altitudes can represent an important source of natural pesticides. Thus, thymol (the compound with highest insecticidal activity) was found in large amounts in essential oils of species that grow at high altitudes (1585-2000 m). In this way we can conclude that places with high altitude are the most suitable for analyzing these species.

For the future the interest in finding more about the essential oil and their pesticide properties from *Thymus balcanus* is important to take into account geographical area and chemical composition of this species. Our studies represent a preliminary stage for developing new strategies in production of biopesticides in order to find new ways for protecting and keeping the environment safe.

Acknowledgement

This paper was published with support provided by the POSDRU/89/1.5/S/49944 project "Developing the innovation capacity and improving the impact of research through post-doctoral programmes".

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Received at: 01. 08. 2012
Revised at: 23. 01. 2013
Accepted for publication at: 29. 01. 2013
Publisged online at: 30. 01. 2013