

PRELIMINARY ASPECTS ON THE PHYTOTOXICITY OF SOME *THYMUS* SPP. AQUEOUS EXTRACTS

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Abstract: Phytotoxic natural compounds enable the development of alternative biocontrol products, therefore increasing numbers of species are screened for identification of such substances. The current paper assesses the phytotoxic potential of aqueous extracts of three *Thymus* species: *T. comosus*, *T. dactyloides* and *T. praecox* against *Raphanus sativus* and *Brassica oleracea* test species. The germination percentage, speed of germination (SG) and accumulated speed of germination (AS) were significantly lowered in both test species, especially by the aqueous macerates tested. Plantlet growth was reduced by macerates treatments in a significant manner, in *R. sativus*, while in *B. oleracea* plantlets growth was significantly reduced by only one macerate and by some of the hydrosols prepared from flowering plants. Observed phytotoxic activity can be assigned to the presence of bioactive secondary compounds in tested extracts, rather than to physico-chemical factors, as indicated by spectrophotometric, pH and conductivity analyses.

INTRODUCTION

Plant secondary metabolites reflect the necessities to defend against attacks from pathogen or predatory organisms as well as against the damaging effects of abiotic factors (excessive light radiation, extreme temperatures, salinity etc.) (Bennett & Wallsgrove, 1994; Wink, 2003; Kennedy & Wightman, 2011). Also the biosynthesis of such compounds may offer an advantage when competing with other plants for resources (Jilani et al., 2008).

Presently over 100.000 plant secondary metabolism products are known, broadly classified in alkaloids, siphimic acid derivatives, terpenoids etc. (Wink, 2009). Among bioactivities of these compounds, the inhibition of growth of neighbouring plants more recently generated interest, such interactions pertaining to the allelopathic phenomenon (Kruse, 2000). The phytotoxic activity of such substances enables the development of natural herbicidal products (Bajwa, 2014). Known examples of this type of compounds are sorgoleone, momilactones A and B, artemisinin (Dayan et al., 1999), leptospermane (Dayan & Duke, 2014), 1,8-cineol and 1,4-cineol (Soltys et al., 2013), which affect the germination, growth, photosynthetic rate, plant transpiration, etc. through metabolic pathway alterations (Lorenzo et al., 2013).

Phytotoxic compounds occur widely within the plant kingdom, being encountered in various families such as Asteraceae, Poaceae, Amaranthaceae, Lamiaceae etc. Within the *Thymus* genus, of identified bioactive compounds, thymol and carvacrol are the most significant ones, followed by p-cymene, γ-terpinene, borneol, terpinene-4-ol și 1,8 cineol (Maksimović et al., 2008; Stahl-Biskup, 2011). Also, a high number of polyphenols, especially rosmarinic acid, chlorogenic and caffeic acid, along epicatechin and ferulic acid etc. were identified within the species of the genus (Boros et al., 2010).

Studies regarding the phytotoxic activity of species belonging to *Thymus* genus (Lamiaceae family) revealed the prominent role played by terpenoidic compounds. Terpenoids synthesized by these plants are known to have a certain influence on neighbouring plants development, with evolutionary adaptations being identified due to interactions with *Thymus* plants. Moreover, different chemotypes of *T. vulgaris* may generate different responses from interacting plants, further emphasizing the major role of the chemical origin of the compounds in allelopathy (Thorpe et al., 2011). It is assumed that such compounds reach the soil as a result of rain, which leaches the volatile compounds out of glandular structures found on leaves and stem surfaces. Meanwhile, shedded leaves further lead to the accumulation of bioactive compounds in the substrate. Once in the soil, these substances may generate phytotoxicity either in a raw state or as a result of microbial transformations (Linhart et al., 2014). Under natural conditions, allelopathic activity was identified in several *Thymus* species such as *T. vulgaris*, *T. pulegioides* and *T. serpyllum* (Thorpe et al., 2011), activity supposedly assigned to phenolic and nonphenolic monoterpenes synthesized in these plants (Callaway, 2010). Phytotoxic activity was proven in soil experiments for some *T. vulgaris* chemotypes (Linhart et al., 2014) as well as *in vitro* for various types of extracts (aqueous, alcoholic, etheric) or for volatile oils of *T. capitatus* and *T. vulgaris* (Hemada & El-Darier, 2011), *T. numidicus* (Ben El Hadj Ali et al., 2014), *T. serpyllum* (Yan, 2008), *T. vulgaris* (Arouiee et al., 2010), *T. kotschyanus* (Safari et al., 2010; Farajollahi et al., 2012; Gholinejad et al., 2012, Soliman & Zatout, 2014) and *T. caramanicus* (Bagheri & Arjomand Tajadini, 2011). For other species of the genus such studies are not available, but one can assume the presence of secondary

metabolites with similar modes of action due to phylogenetic proximity and of evolutionary acquired traits (Imatomi et al., 2013).

In the Romanian spontaneous flora, 17 *Thymus* species are found in all altitudinal levels, of which *T. dactyloides* and *T. comosus* may be encountered in grasslands and rocky meadows, while *T. praecox* inhabits crystalline rocks habitats (Ciocarlan, 2009).

The current paper focuses on testing aqueous extracts (macerates and aqueous fractions of hydrodistillation) of spontaneous *Thymus* species (*T. dactyloides*, *T. comosus*, *T. praecox*) plants from Romanian flora, aiming to assess the phytotoxic potential of aforementioned species.

MATERIAL AND METHODS

Material

Plant material was represented by aerial parts of *Thymus comosus* Heuff. ex Griseb. & Schenk, *Thymus dactyloides* Borbás and *Thymus praecox* ssp. *polytrichus* (A. Kern. ex Borbás) Jalas. plants, collected in both vegetative as well as flowering development phases. Plant material was harvested from Lotrului Valley, Vâlcea County (*T. dactyloides*), Jina, Sibiu County (*T. comosus*) and Parâng Mountains, Gorj County, alt. 950 m (*T. praecox*), Romania. Prior to the extraction, the plants were dried in ambient conditions (22–24° C temperatures), protected from direct sunlight. The identity of the species was established by prof. PhD Ștefan N., “Alexandru Ioan Cuza” University, Iași, Romania. Vouchers were deposited for each species at the Faculty of Biology Herbarium, “Alexandru Ioan Cuza” University, Iași, Romania.

Extract preparation

Generally, for phytotoxic activity testing, water is preferred as the extraction media, as it has virtually no toxicity, it implies low costs and the extracts require no further processing for use. Two methods were employed for extract preparation: water maceration (5 g plant material in 95 ml tap water) under continuous mixing on a magnetic stirrer, for 4 hours, at room temperature, the extracts subsequently being filtered using Whatman no.1 paper; hydrodistillation (according to European Pharmacopoeia) using a NeoClevenger type apparatus (with water as solvent in a 4:1 ratio to the amount of plant material) for 3 hours, collecting the resulted aqueous fraction (hydrosol). The aqueous fractions contain a series of volatile compounds as a result of the contact of the distilled water in the capillary of the apparatus with the volatile oils.

Phytotoxic activity testing

For the phytotoxic assessment of the extracts, the inhibition of germination and of initial plantlet growth were evaluated in *Raphanus sativus* L. var. *sativus*, Rodica cultivar and *Brassica oleracea* L. var. *capitata* f. *alba* DC, Dittmarscher cultivar, obtained from local seed retailers. The germination and root and stem elongation are frequently used parameters in assessing phytotoxic activity (Hoagland & Williams, 2004). Initially, 25 seeds of each species were placed on filter paper in Petri dishes (9 cm diameter). The filter paper was moistened with 3 ml of extract or, respectively, distilled water in control plates. Seed germination and plantlet growth was performed using a Snijders Scientific ECD 109E growth chamber with a 12/12 h photoperiod, at a 22–24°C temperature.

Testing was made in 3 replicates for each extract and, respectively, controls. The number of germinated seeds was recorded at 24, 48, 72 h after the placement of seeds on the wet filter paper. Root and stem lengths were measured at 18 plantlets for each experimental variant, after 72 h from the initiation of the experiment. The filter paper was maintained moist during the experiment by adding the required quantities of water or extract.

Qualitative analysis of extracts

Obtained extracts were spectrophotometrically evaluated for the presence of chemical compounds. For each aqueous macerate, respectively, hydrosol, absorption spectra were obtained within the 190–1100 nm range, using a Beckman DU730 spectrophotometer and plastic cuvettes with a light path length of 10 mm. Also, pH and electrical conductivity were measured for each extract using a Consort C532 multimeter.

Statistical analysis

The values recorded for seed germination and plantlet growth are expressed as mean values \pm standard errors. The statistical significance of the degree of inhibition was calculated using ANOVA ($p=0,01$) and post-hoc Dunnett test by means of GraphPad Prism 6.0 software.

The extracts treatment effects on seed germination was assessed calculating the: germination percentage (GP), speed of germination (S), speed of accumulated germination (AS) and coefficient of the rate of germination (CRG). The equations of these indices are described in papers concerning the effects of allelopathic compounds on test plants, considering that they adequately reflect the course of the germination process (Chiapusio et al., 1997; Anjum & Bajwa, 2005; Islam & Kato-Noguchi, 2014).

RESULTS AND DISCUSSIONS

The influence of the treatments on germination

In the case of *Raphanus sativus* seeds, the germination percentages were significantly lowered, compared to the control, by all aqueous macerates (80,96% – 95,25%) (Table 1) and hydrosols (84,21% - 94,75%) (Table 2) treatments after 24 hours from the beginning of the experiment. Significantly lower germination percentages were recorded after 72 hours only in the case of three aqueous macerates (*T. praecox* anthesis, *T. dacicus* vegetative and *T. dacicus* anthesis). The speed of germination (SG) and the accumulated speed of germination (AS) were lowered more by the treatments with aqueous macerates than the ones with hydrosols comparing with the controls. The coefficient of the rate of germination was the least affected germination index following the treatments (Figure 1).

Table 1. Germination percentages in *Raphanus sativus* seeds (control and aqueous extract treatments, means ± standard error, a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

| | <i>T. praecox</i> vegetative | <i>T. praecox</i> anthesis | <i>T. dacicus</i> vegetative | <i>T. dacicus</i> anthesis | Control |
|-------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|---------|
| 24 h | 5.33 ^d ±2.31 | 1.33 ^d ±2.31 | 1.33 ^d ±2.31 | 1.33 ^d ±2.31 | 28±4 |
| 48 h | 28 ^b ±8 | 26.67 ^b ±4.62 | 22.67 ^c ±2.31 | 18.67 ^c ±11.55 | 56±4 |
| 72 h | 41.33±6.11 | 34.67 ^a ±4.62 | 30.67 ^b ±15.14 | 38.67 ^a ±4.62 | 60±6.93 |

Table 2. Germination percentages in *Raphanus sativus* seeds (control and hydrosol treatments; means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

| | <i>T. praecox</i> vegetative | <i>T. praecox</i> anthesis | <i>T. dacicus</i> vegetative | <i>T. dacicus</i> anthesis | <i>T. comosus</i> vegetative | <i>T. comosus</i> anthesis | Control |
|-------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|-------------|
| 24 h | 1.33 ^c ±2.31 | 14.67±8.33 | 4 ^b ±4 | 2.67 ^b ±2.31 | 2.67 ^b ±4.62 | 4 ^b ±4 | 25.33±10.07 |
| 48 h | 65.33±12.22 | 50.67±18.9 | 56±17.44 | 56±16 | 64±17.44 | 74.67±10.07 | 70.67±6.11 |
| 72 h | 72±14.42 | 50.67±16.17 | 64±16 | 70.67±12.86 | 72±10.58 | 80±13.86 | 81.33±8.33 |

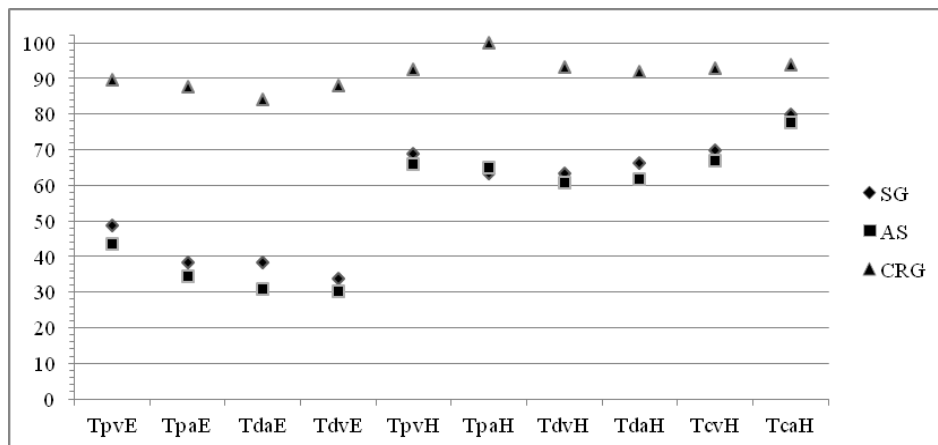


Figure 1. Speed of germination (SG), accumulated speed of germination (AS) and coefficient of the rate of germination (CRG) for *R. sativus* seeds treated (% from control) (T.p. – *Thymus praecox*; T. d. – *Thymus dactylicus*; T. c. – *Thymus comosus*; v – vegetative phase; a – anthesis phase; E – aqueous macerate; H – hydrosol)

For *Brassica oleracea* seeds, the germination percentages were significantly reduced only following the treatments with aqueous macerates (Table 3), although lower values were recorded in the case of some hydrosols (Table 4), without being statistically significant. After 72 hours from the beginning of the experiment, the inhibition of germination was significant for the same three macerates which reduced the germination in *Raphanus sativus* seeds after the same time interval. The speed of germination (SG) and the accumulated speed of germination (AS) presented lower values compared to the controls in the case of all aqueous macerates. Only the hydrosols obtained from plants in the anthesis stage induced a reduction of these two indices, while hydrosols from vegetative stage plants stimulated germination. The coefficient of the rate of germination was, as in the case of radish seeds, the least sensible index (Figure 2).

Table 3. Germination percentages in *Brassica oleracea* seeds (control and aqueous extract treatments; means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

| | <i>T. praecox</i> vegetative | <i>T. praecox</i> anthesis | <i>T. dactylicus</i> vegetative | <i>T. dactylicus</i> anthesis | Control |
|-------------|---------------------------------|-------------------------------|------------------------------------|----------------------------------|-------------|
| 24 h | 0±0 | 1,33±2,31 | 0±0 | 0±0 | 0±0 |
| 48 h | 17,33 ^b ±16,17 | 22,67 ^a ±22,03 | 13,33 ^b ±4,62 | 22,67 ^a ±4,62 | 62,67±12,86 |
| 72 h | 53,33±8,33 | 38,67 ^a ±22,03 | 33,33 ^b ±16,17 | 45,33 ^a ±6,11 | 81,33±10,07 |

Table 4. Germination percentages in *Brassica oleracea* seeds (control and hydrosol treatments; means \pm standard error)

| | <i>T. praecox</i> vegetative | <i>T. praecox</i> anthesis | <i>T. dacticus</i> vegetative | <i>T. dacticus</i> anthesis | <i>T.</i> <i>comosus</i> vegetative | <i>T.</i> <i>comosus</i> anthesis | Control |
|---------|---------------------------------|-------------------------------|----------------------------------|--------------------------------|---|---|-------------------|
| 24 h | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 |
| 48 h | 53.33 \pm 11.55 | 30.67 \pm 10.07 | 60 \pm 4 | 24 \pm 41.57 | 68 \pm 8 | 16 \pm 16 | 41.33 \pm 11.55 |
| 72 h | 72 \pm 8 | 60 \pm 12 | 73.33 \pm 12.86 | 54.67 \pm 36.07 | 88 \pm 4 | 56 \pm 20 | 64 \pm 6.93 |

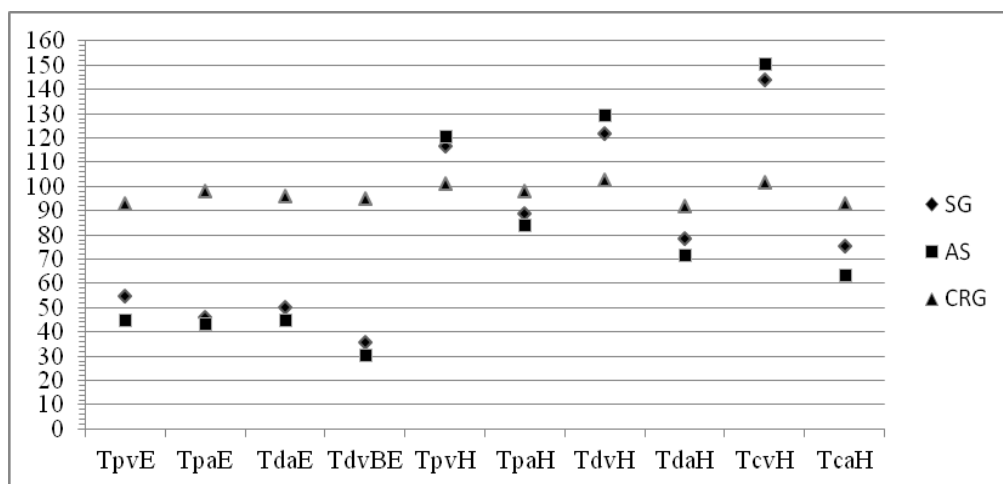


Figure 2. Speed of germination (SG), accumulated speed of germination (AS) and coefficient of the rate of germination (CRG) for *B. oleracea* seeds treated (% from control) (T.p. – *Thymus praecox*; T. d. – *Thymus dacticus*; T. c. – *Thymus comosus*; v – vegetative phase; a – anthesis phase; E – aqueous macerate; H – hydrosol)

The influence of the treatments on plantlet growth

For *Raphanus sativus* plantlets, a significant reduction in root and stem elongation was induced by all the treatments with aqueous macerates (Table 5 (a)), the *T. dacticus* extracts being generally more powerful than *T. praecox* ones. The hydrosol treatments did not significantly reduce the growth of radish plantlets, instead, the hydrosols obtained from vegetative stage plants stimulated the stem elongation (Table 5 (b)).

In the case of *Brassica oleracea* plantlets, a significant reduction of growth was observed only for *T. dacticus* aqueous macerate (Table 5 (a)), which reduced root length, the *T. dacticus*

anthesis hydrosol, which reduced stem length and *T. comosus* anthesis hydrosol, that reduced both root and stem elongation (Table 5 (b)).

Table 5 (a). Root and stem length (cm) in control and aqueous macerates treatments (means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

| Aqueous macerate | <i>T. praecox</i> vegetative | <i>T. praecox</i> anthesis | <i>T. dacicus</i> vegetative | <i>T. dacicus</i> anthesis | Control |
|--------------------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|------------|
| <i>R. sativus</i> root length | 9.06^b±0.83 | 8.94^b±1.25 | 6.61^d±0.68 | 8.61^b±0.88 | 14.33±1.62 |
| <i>R. sativus</i> stem length | 4.44^d±0.40 | 5.72^c±0.81 | 4.28^d±0.47 | 4.50^d±0.36 | 9.11±0.80 |
| <i>B. oleracea</i> root length | 5.94±0.66 | 5.22±0.56 | 4.33±0.55 | 3.56^c±0.57 | 6.94±0.64 |
| <i>B. oleracea</i> stem length | 2.83±0.29 | 3.00±0.33 | 2.61±0.29 | 2.39±0.20 | 3.06±0.26 |

Table 5 (b). Root and stem length (cm) in control and hydrosols treatments (means ± standard error; a: p≤0,05; b: p≤0,01; c: p≤0,001; d: p≤0,0001)

| Hydrosol | <i>T. praecox</i> vegetative | <i>T. praecox</i> anthesis | <i>T. dacicus</i> vegetative | <i>T. dacicus</i> anthesis | <i>T. comosus</i> vegetative | <i>T. comosus</i> anthesis | Control |
|--------------------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|------------|
| <i>R. sativus</i> root length | 17.22±5.71 | 13.50±5.85 | 14.67±5.59 | 13.61±4.00 | 18.06±6.78 | 17.17±5.59 | 17.72±6.13 |
| <i>R. sativus</i> stem length | 10.11^d±1.68 | 7.83±1.76 | 9.33^b±1.91 | 8.33±1.91 | 10.39^d±1.72 | 8.00±1.68 | 7.44±1.69 |
| <i>B. oleracea</i> root length | 7.89±2.19 | 7.06±2.56 | 9.11±4.20 | 5.00±2.43 | 10.94±3.98 | 4.00^c±1.82 | 8.22±3.81 |
| <i>B. oleracea</i> stem length | 3.11±0.83 | 3.11±0.96 | 3.44±1.20 | 2.56^c±1.89 | 3.33±1.09 | 1.39^d±1.29 | 4.22±1.26 |

Physico-chemical characteristics of the extracts

The pH values of the aqueous macerates indicate a more acid character compared to the initial extractive media (water). For hydrosols, the pH had higher values (alkaline character) than the distilled water used for extraction. Conductivity values show an increased concentration of mineral elements compared to the initial extraction media, in both aqueous macerates and hydrosols, with higher values for the macerates (Table 6).

Table 6. Conductivity and pH values for extraction media and extracts (T.W. – tap water; D.W. – distilled water; T.p. – *Thymus praecox*; T. d. – *Thymus dacicus*; T. c. – *Thymus comosus*; v – vegetative phase; a – anthesis phase; E – aqueous macerate; H – hydrosol)

| Solution | T.W. | D.W. | TPvE | TPaE | TDvE | TDaE | TPvH | TPaH | TDvH | TDaH | TCvH | TCaH |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| pH | 7.70 | 6.46 | 6.61 | 6.48 | 6.48 | 6.29 | 9.49 | 8.30 | 9.29 | 8.80 | 7.26 | 7.02 |
| Conductivity (mS) | 0.36 | 0.01 | 1.55 | 2.55 | 1.59 | 1.72 | 0.17 | 0.13 | 0.24 | 0.09 | 0.06 | 0.04 |

The absorption spectra indicate the presence of different types of compounds in the tested extracts (Figure 3, 4). The high values of absorbances in the 240-260 nm region possibly signify

phenolic compounds which usually have an absorption maxima (K bands) at 254 nm. Meanwhile, peaks in the 270-280 nm region are characteristic to different types of ethers. Also, the presence of polycyclic aromatic compounds may be assumed from the peaks in 320-350 nm area. However, one must take into account the polarity of the solvent used (water) which usually induces an absorbance shift towards higher wavelengths (red shift) of $\pi \rightarrow \pi^*$ transitions and towards lower wavelengths (blue shift) of $n \rightarrow \pi^*$ (Kalsi, 2004; Pretsch et al., 2009; Kaye & Laby, 2005). The recorded absorbances indicate a higher concentration of phenolic compounds in the macerates than in hydrosols.

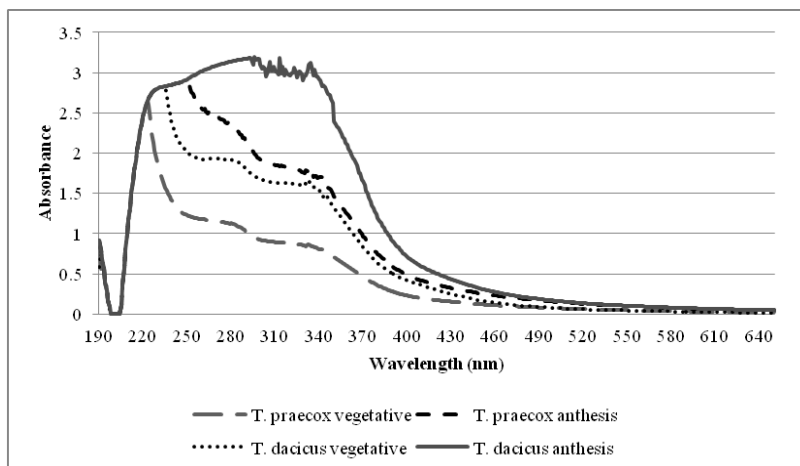


Figure 3. Absorbance spectra of aqueous macerates

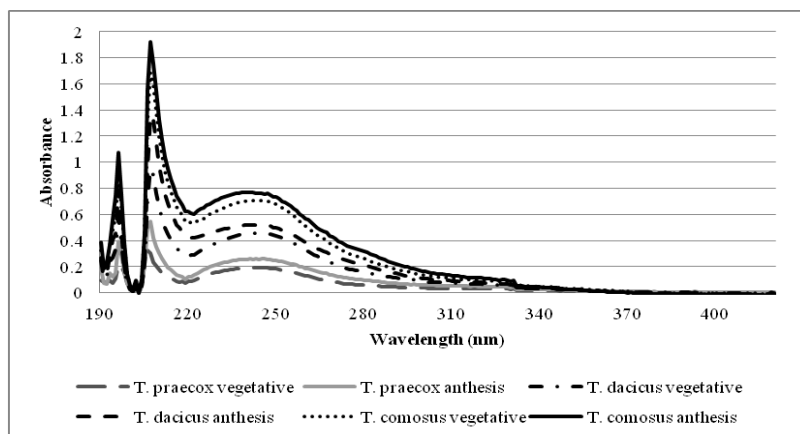


Figure 4. Absorbance spectra of hydrosols

The inhibitory effects of treatments with various extracts on germination and plantlet growth of tested species might be assigned to the compounds present in these extracts, presumably

phenolics and terpenoids, as observed from the spectrophotometric analyses.

The phytotoxic activity is known for phenolic compounds, which determine the inhibition of germination in 10^{-3} to 10^{-5} M concentrations (Williams & Hoagland, 1982). Such phytotoxicity is induced by various mechanisms such as alterations in membrane permeability, nutrient uptake reduction, inhibition of cell division and cellular elongation, lowering of photosynthetic and respiration rates, alterations in enzymatic activities, endogenous phytohormones and protein synthesis rate reduction (Li et al., 2010). Similarly, terpenoids are potent inhibitors of the germination and growth of plants, with more powerful effects in the case of monoterpenes and sesquiterpenes (Macias et al., 1999). Terpenoids inhibitory activities are due to mitochondrial respiration inhibition, plasmatic membrane permeability alteration, inhibition of chlorophyll and hormone synthesis etc., however, the modes of action being known for relatively few compounds (Duke & Oliva, 2004).

Testing for phytotoxic activity must account for pH and conductivity of extracts in order to discriminate between effects of actual compounds and those of different values of osmolarities and acidities than normal, physiological ones present in cells and tissues of test plants (Hoagland & Williams, 2004; Dayan & Duke, 2005).

Optimal pH values for germination of some cultivated species are between 6.5 – 6.7, with no significant effects of higher values, but with inhibition occurring at values below 6.5 (Pérez-Fernández et al., 2006; Deska et al., 2011). For the tested *Thymus* extracts, the values of the pH ranged between 6.29 – 6.61 (aqueous macerates) and 7.02 – 9.49 (hydrosols), allowing thus the assumption that this parameter did not significantly influence the germination process.

The conductivity of solutions is an indicator of ionic concentration, a factor that may lead to an inhibitory effect due to a hyper or hypotonic media. The conductivity values of the tested *Thymus* extracts varied between 0.24 mS (hydrosols) and 2.55 mS (aqueous macerates), which are lower than inhibitory values of 4 – 8 mS as determined for some cultivated plant species (Pendleton & Meyer, 1990; Mer et al., 2000).

The degree of phytotoxicity varies among the tested extracts. Regarding the aqueous macerates, in both *Raphanus sativus* and *Brassica oleracea* seeds, the most pronounced inhibitory effect on the germination process is observed for the extracts with the highest amounts of compounds as seen in the corresponding absorption spectra. On the other hand, the most active hydrosols concerning inhibition of plantlet growth of both test species are those obtained from vegetative stage plants of the three *Thymus* species. The inhibitory effect of hydrosols on organ elongation is in an inverse relation with the amount of compounds observed in the spectrophotograms, suggesting thus that the nature and not the amount of compounds determines the phytotoxicity. The variation of the chemical composition of volatile oils depending on the ontogenetic phase is known in many plants, including *Thymus* species where the anthesis is accompanied by a richer compound variety and concentration (Jordan et al., 2006; Gallaso et al., 2014). Generally, different compounds are attributed different phytotoxicities, for *Thymus* species, the carvacrol and thymol being considered the most active substances (Tarayre et al., 1995; Morales, 2002).

The effectiveness of the treatments varied with the type of extract used, generally the germination process being inhibited to a larger extent by the aqueous macerates compared to the hydrosols. The same observation stands also for the growth of *Raphanus sativus* plantlets, where some hydrosols even determined a stimulatory effect.

Several studies underlined the allelopathic activity of some *Thymus* species in natural environments and demonstrated such effects under controlled conditions, as germination and plant

growth was inhibited by several extracts or by individual terpenoidic compounds (Vokou et al., 2003; Angelini et al., 2003; Hemada & El-Darier, 2011; Ben El Hadj Ali et al., 2014). The obtained results reveal similar activities for the *T. dacicus*, *T. comosus*, *T. praecox* species, complementing previous studies.

CONCLUSIONS

The tested extracts exert a certain degree of phytotoxicity towards the two test species used. A higher inhibitory effect on germination (for both test species) was identified for the aqueous macerates (5% concentration). The growth of the plantlets was significantly reduced by the aqueous macerates for *Raphanus sativus*, while *Brassica oleracea* was less sensible to the treatments. Considering the pH and conductivity of the tested extracts, the inhibitory effects on germination and growth might be attributed to the compounds present rather than to the physico-chemical properties of the treatments. Because there are relatively few results concerning the phytotoxicity of the investigated *Thymus* species, further testing of various concentrations of extracts is required.

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