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Comparative Study on Composition and Biological Activity of Essential Oils of Two *Thymus* Species Grown in Egypt

M. Hemada and S. El-Darier

Department of Botany and Microbiology,
Faculty of Science, Alexandria University, Alexandria, Egypt

Abstract: The present study aimed to evaluate the chemical composition of the essential oils extracted from the dry shoots of *Thymus capitatus* L. *and Thymus vulgaris* L. and their allelopathic potentiality using growth bioassays of seeds of *Lepidium sativum* L. (garden cress) and *Raphanus sativus* L. (radish). Hydro-distilled essential oils from the two thyme species were obtained in yield of about 2.1 and 1.4% (v/w), respectively based on dry weight basis. The major compounds identified in *T. capitatus* and *T. vulgaris* represented percentages of about 94 and 83%, respectively. Conveniently, the oil components can be grouped into four major classes: hydrocarbon monoterpenes (HM), oxygenated monoterpenes (OM), monoterpene esters (ME) and monoterpene alcohols (MA). The two types of thyme oil exerted an allelopathic effect on the different germination parameters (GP, T_{50}, MGT, SGI and hypocotyl (HL) and radicle (RL) lengths of the two test species. It is important to mention that *R. sativus* seeds were highly resistant for oil application as compared with *L. sativum,* which are the most sensitive.

Key words: *Thymus capitatus* · *Thymus vulgaris* · Allelopathy · Monoterpenes · Germination parameters · Essential oils

INTRODUCTION

The genus *Thymus* L. consists of about 350 species of perennial, aromatic herbs and sub- shrubs native to Europe, North Africa and Asia. Various types of thyme are used all over the globe as condiments, ornamentals and sources of essential oil. Thyme oil is among the world's top ten essential oils, displaying antibacterial, antimycotic, antioxidative, food preservative and mammalian age-delaying properties [1]. Thyme is best cultivated in a hot sunny location with well drained soil. It is generally planted in the spring and thereafter grows as a perennial. It can be propagated by seed, cuttings, or by dividing rooted sections of the plant and tolerates drought well [2]. Thyme retains its flavor on drying better than many other herbs. The plant can be used fresh at any time of the year, or it can be harvested as it comes into flower and either is distilled for the oil or dried for later use. The essential oil should not be used in aromatherapy because it is highly irritant to the mucous membranes [3]. Medicinally, thyme is used for respiratory infections (in the form of a tincture, tisane, salve, syrup or by steam inhalation) inflammation of the throat and for uterine contractions for more rapid delivery of the placenta [4].

Thymus capitatus L. (wild thyme) and *Thymus vulgaris* L. (Garden thyme) are two common widely distributed thyme species recorded in Egypt. *Thymus capitatus* is a densely glandular-dotted dwarf shrub with small entire leaves and flowers arranged in terminal heads. It is mostly common in Egypt in rocky habitats. Pure essential oil derived from *Thymus capitatus* contains about 65% carvacrol. *Thymus vulgaris* (Garden thyme) is cultivated for its strong flavor, which is due to its content of thymol (20-55%) [4]. Thymol, an antiseptic, is the main active ingredient in different mouthwashes [5]. Before the advent of modern antibiotics, it was used to medicate bandages [6]. It has also been shown to be effective against the fungus that commonly infects toenails [7]. Despite the abovementioned uses; thyme plant is defined as being able to alter the structure and function of native communities [8]. The occurrence of deleterious biochemical interactions among higher plants is generally considered a significant ecological factor in determining

Corresponding Author: S. El-Darier, Botany and Microbiology Department,
Faculty of Science, Alexandria University, Alexandria, Egypt.

the structure, variety and composition of plant communities [9]. Allelopathic chemicals are generally considered to be secondary plant products which are released directly from living plants into the environment via leaching, root exudation, volatilization, or the decomposition of plant residues [10, 11]. *Thymus* species as well as many other aromatic plants biosynthesize remarkable amount of volatile compound referred as the essential oil, therefore chemical classification and action of such plants is based on the main essential oil components [12]. Most chemicals that have been identified in allelopathic interactions have been identified as either terpenes or phenolic compounds [13]. Also components from essential oils may inhibit seed germination as well as plant growth. Some of the essential oils considered to exert allelopathic effects can be extracted from *Tagetes minuta* L. *Schinus areira* L. [9], *Ruta graveolens* L. [14], *Rosmarinus officinalis* L. *Thymus vulgaris* L. *Satureja montana* L. [15] and *Conyza albida* Wild. [16].

The present study aimed to compare the chemical composition of the essential oils extracted from wild and garden thyme and to evaluate the possibility of interrelationship between oil characteristics and allelopathic potentiality using germination bioassays of seeds of *Lepidium sativum* L. (garden cress) and *Raphanus sativus* L. (radish).

MATERIALS AND METHODS

Plant Materials: The shoots of *Thymus capitatus* and *Thymus vulgaris* were collected during summer 2010 from natural and cultivated fields found in rocky ridges at Burg El-Arab and at El-Sharkeyya Governorate, Egypt, respectively. Plants were identified and authenticated according to Täckholm [17] and Boulos [18]. A voucher specimen was deposited in the herbarium of Botany and Microbiology Department, Faculty of Science, Alexandria University.

Extraction of the Volatile Oils: The essential oil was extracted from dried aboveground shoots of the two plant species (100 g) by hydro-distillation method for three hours using Clevenger-type apparatus. The extracted oils were dried over anhydrous sodium sulphate and the oil content was determined in triplicate and the mean values were determined [19]. The collected oils were subjected to GC/MS investigation using Hewlett-Packard GC/MS Spectrometer, model 5970 with the following conditions: Fused silica capillary column (50 m x 0.32 mm) coated with Carbowax 20M. Oven temperature was programmed; 60-200°C increased by 3°C /min. Helium carrier gas with flow rate of 1 ml/min. Injection temperature 150°C, TIC detector. MS ionization voltage 70 ev. Qualitative and quantitative identification of the oil constituents were carried out by comparing the retention times and mass fragmentation with computer matching, as well as retention indexes and the previously published data.

Germination and Growth Bioassay: Bioassays were carried out with seeds of *Lepidium sativum* L. (garden cress) and *Raphanus sativus* L. (radish) as bioassay materials (test species). Essential oils solutions were prepared at concentrations of 1,000, 5,000 and 10,000 μg ml^{-1} in dichloromethane. Assays were conducted in 9-cm diameter glass Petri-dishes lined with 1 sheet of Whatman No. 1 filter paper and sealed with Parafilm. Three ml of each solution was added to each dish and the solvent was evaporated before addition of 10 ml of water followed by 20 seeds of *L. sativum* or *R. sativus*. Before sowing, the seeds were surface sterilized by soaking for two minutes in 4% sodium hypochlorite, then, rinsed four times with distilled water. Assays were carried out at 25°C under artificial fluorescent light (8 x 40 W) in an incubator for 3 days. Seeds were considered to have germinated if a radicle protruded at least 1 mm. A control experiment was carried out under the same conditions described but using only water instead of the test oil. Each bioassay was replicated 3 times in a complete randomized design. Data respecting germination percentage (GP) and hypocotyl (HL) and radicle (RL) lengths were recorded after three days. The time to get 50% germination (T_{50}), the mean germination time (MGT), seed germination index (SGI), energy of germination (GE) and phytotoxicity (PT) were calculated according to the following equations:

The time to get 50% germination (T_{50}) was calculated according to the following formula of Coolbear *et al.* [20] modified by Farooq *et al.* [21].

$$T_{50} = t_i\ (N/2 - ni)\ (t_j - t_i)/n_j - n_i$$

Where N is the final number of germinated seeds and n_j, n_i are the cumulative number of seeds germinated by adjacent counts at times t_j, t_i when $ni < N/2 < n_j$.

The mean germination time (MGT) was calculated according to the equation of Battle and Whittington [22].

$$MGT = \Sigma(G \times T) / F$$

Where:

T = The day on which germination count was made
G = The number of seeds germinated on the day of the count
F = Final number of seeds which germinated in each replicate

Seed germination index (SGI) was calculated according to the equation of Scott *et al.* [23].

$$SGI = \Sigma\ Ti\ Ni/S$$

Where,

Ti = Is the number of days after sowing
Ni = Is the number of seeds germinated on day i
S = Is the total number of seeds planted

Energy of germination (GE) was recorded according to Farooq *et al.* [21] at the 4th day after sowing. It is the percentage of germinating seeds (GP) four days after sowing relative to the total number of seeds tested (TNST).

$$GE = GP\ (4^{th}\ day)/TNST$$

Likewise, phytotoxicity was calculated according to the general equations:

Phytotoxicity (PT) = [1- (allelopathic/control) 100]

Statistical Analysis: Data concerning the effect of different concentrations of thyme volatile oils on germination percentage (GP), the time to get 50% germination (T_{50}), the mean germination time (MGT), hypocotyl (HL) and radicle (RL) lengths were subjected to standard one-way analysis of variance (ANOVA) according to Zar [24] using the COSTAT 2.00 statistical analysis software manufactured by Cohort software company.

RESULTS

Chemical Composition of Thyme Essential Oils: Hydro-distilled essential oils from the dry shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* (TV) has been analyzed by GC/MS. The major compounds identified are given in Table 1. The essential oils were found to be more or less a pale yellow liquid, obtained in yield of about 2.1 and 1.4% (v/w) for *T. capitatus* and *T. vulgaris*, respectively based on dry weight basis. The major compounds identified in the essential oil of *T. capitatus* and *T. vulgaris* are α- thujene (0.70 & 0.46%), α-pinene (4.3 & 3.35%), myrcene (0.92 & 0.87%), α-terpinene (2.99 & 2.81%), p-cymene (4.19 & 3.63%), sabiene (4.3 & 2.1%), para-menthene-1 (2.9 & 1.8%), limonene (0.9 & 0.6%), 1,8-cineol (2.61 & 2.54%), thymol (15.17 & 12.74%) and carvacrol (53.16 & 48.23%), respectively. Prominently, the compounds, α-thujene, α-pinene, α-terpinene, Sabiene, myrcene and p-cymene which are considered the most important monoterpenes hydrocarbons are higher in

Table 1: Chemical composition of the essential oils of the dry shoots of *Thymus capitatus*(TC) and *Thymus vulgaris* (TV).

Group	Compound	TC (%)	TV (%)
Monoterpene hydrocarbons	p-Cymene	4.19	3.63
	α-Terpinene	2.99	2.81
	α-pinene	4.30	3.35
	Myrcene	0.92	0.87
	Camphene	0.40	0.30
	Sabiene	4.30	2.10
	Limonene	0.90	0.60
	α-thyjene	0.70	0.46
Oxyganated phenolic monoterpenes	Thymol	15.17	12.74
	Carvacrol	53.16	48.23
Monoterpene esters	1,8-cineol	2.61	2.54
Monoterpene alcohols	1,3-Octadiene	0.50	0.30
	1,7-octadiene	0.20	0.10
	Borneol	5.30	4.92
Other compounds	Para-menthene-1	2.90	1.8
	Para-menthene-1	0.30	0.10
Total	93.84	82.75	

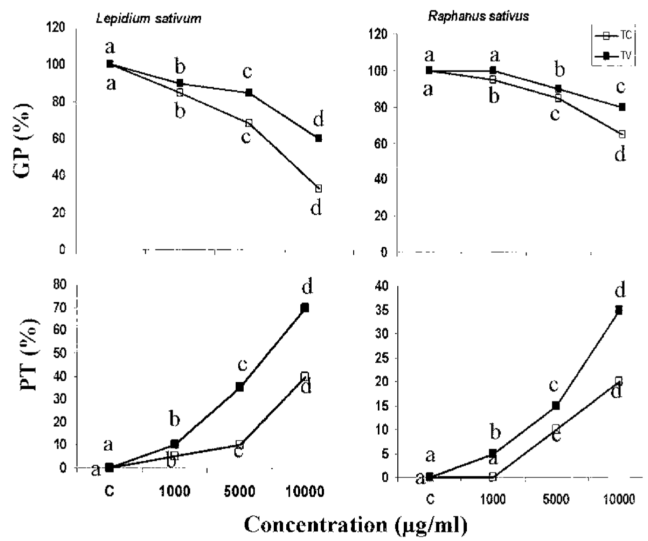

Fig. 1: Effect of volatile oil extracted from the dry shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* (TV) on germination percentage (GP) and phytotoxicity (PT) of *Lepidium sativum* and *Raphanus sativus* seeds. (Values are the mean of three replicates. Different letters for each line indicate a significant difference at probability level = 0.05 according to ONE-WAY ANOVA test).

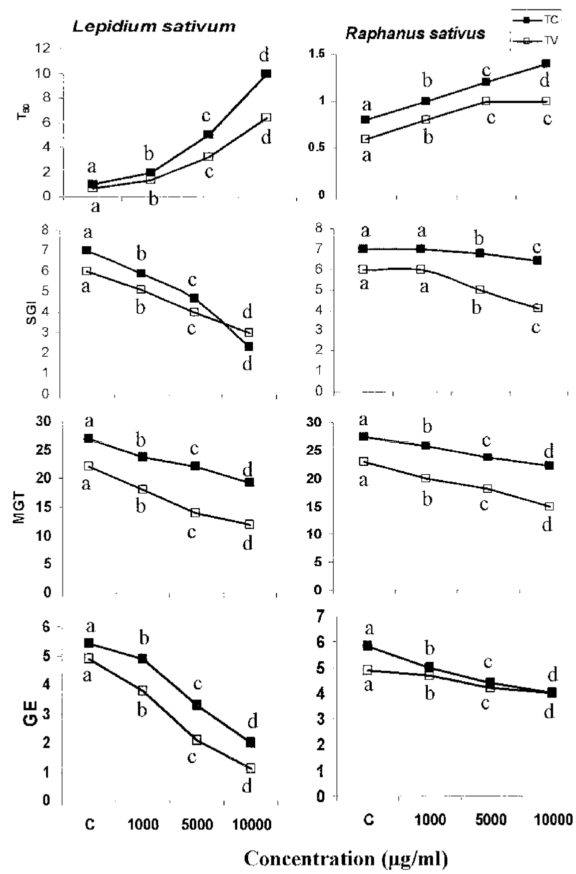

Fig. 2: Effect of volatile oil extracted from the dry shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* (TV) on 50% germination (T50), seed germination index (SGI), mean germination time (MGT) and germination energy (GE) of *Lepidium sativum* and *Raphanus sativus* seeds. (Values are the mean of three replicates. Different letters for each line indicate a significant difference at probability level = 0.05 according to ONE-WAY ANOVA test).

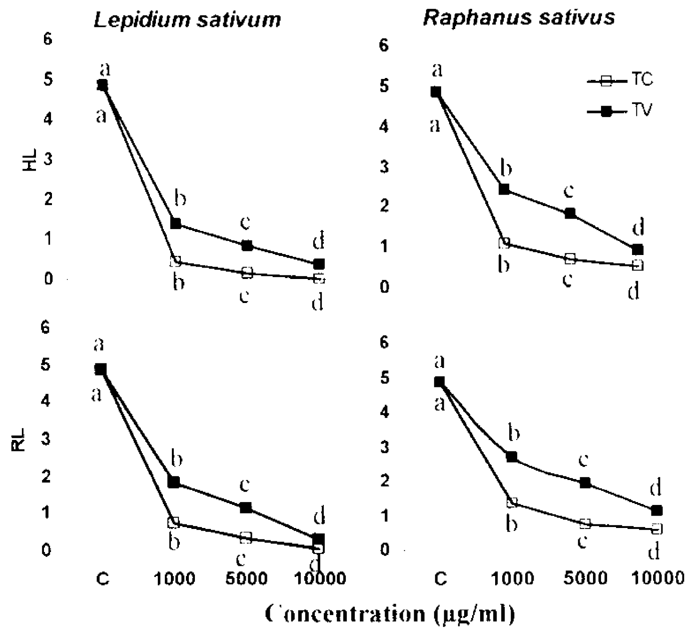

Fig. 3: Effect of volatile oil extracted from the dry shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* (TV) on hypocotyl (HL) and radicle (RL) length of *Lepidium sativum* and *Raphanus sativus* seeds. Values are the mean of three replicates. Different letters for each line indicate a significant difference at probability level = 0.05 according to ONE-WAY ANOVA test.

T. capitatus compared to *T. vulgaris*. The major compounds identified in *T. capitatus* and *T. vulgaris* represented a percentage of about 94 and 83%, respectively. Conveniently, the oil components can be grouped into four major classes: hydrocarbon monoterpenes (HM), oxygenated monoterpenes (OM), monoterpene esters (ME) and monoterpene alcohols (MA). The oxygen-containing phenolic monoterpenes, carvacrol and thymol were found to be the major constituents in the essential oil of the two thyme species, followed by moderate amounts of the monoterpenic alcohols (borneol), while the monoterpenic hydrocarbons (p-cymene, α-pinene) came up to the third level.

Germination Bioassay: The effect of essential oils extracted from the dry shoots of *Thymus capitatus* and *Thymus vulgaris* was evaluated upon germination percentages (GP) of *Lepidium sativum* L. (garden cress) and *Raphanus sativus* L. (radish) (Fig. 1). Commonly, GP decreased with increasing essential oil concentration. At the end of the experiment (after three days) GP attended a value of about 100 % at control level for both the two target thyme species and the two test species. GP of *L. sativum* achieved a continuous decrease to reach minimum values of about 33 and 60% for *T. capitatus* and *T. vulgaris*, respectively at the maximum oil concentration (10^4 µg ml^{-1}). The relative records for *R. sativus* were about 65 and 80%, respectively. It is important to mention that *R. sativus* seeds were highly resistant for oil application as compared with *L. sativum,* which are the most sensitive.

Data of the present study demonstrated that the phytotoxicity (PT) increased with the increase of essential oil concentration (Fig. 1). Coming again to note that seeds of *L. sativum* were more sensitive compared to those of *R. sativus* where the PT reached to a value of about 67 and 40% in the former compared to 35 and 20% in the latter for *T. capitatus* and *T. vulgaris*, respectively. Similarly, the time to get 50% germination (T_{50}) increased conspicuously as essential oil concentration increased which is higher in *L. sativum* relative to the other test species (Fig. 2). In addition, the same figure also illustrates the seed germination index (SGI), the mean germination time (MGT) and energy of germination (GE) for the two types of germinated seeds. The values of the three measures decreased distinctly as essential oil concentration increased. Regarding SGI, the decrease was more imperative in *L. sativum* as compared with the other test species. Generally, cultivated thyme was less effective compared to wild thyme. Concerning MGT, the two test species exhibited more or less the same trend. As regards GE, the reduction was most prominent in *L. sativum* compared to *R. sativus*.

Different oil concentrations of the two thyme species exerted an allelopathic effect on hypocotyl (HL) and radicle (RL) lengths of the two test species (Fig. 3). Generally, the two growth parameters were reduced with the increase in oil concentrations. Two similar trends were obtained for *R. sativus* and *L. sativum* and the former was more resistant than the latter. Noteworthy, the effect of wild thyme oil was more prominent compared to cultivated thyme oil. The wild and cultivated thyme oil concentration of 10^3 μg ml^{-1}, severely inhibited hypocotyl (91 and 72% for *L. sativum* and 77 and 50% for *R. sativus* respectively) and radicle (85 and 62% for *L. sativum* and 72 and 44% for *R. sativus,* respectively) relative to the control. However, at the concentration of 10^4 μg ml^{-1}; all samples caused significant inhibition on the hypocotyl and radicle growth of both species and, in general, the two oil samples tested were more active in inhibiting the hypocotyl (89 and 81% for *R. sativus* and 100 and 93% for *L. sativum*, respectively) and radicle growth for *L. sativum* (98 and 94%) than for *R. sativus* (88 and 77%).

DISCUSSION

Several studies have been interested in the biologically active compounds isolated from plant species for food preservation [25], the elimination of pathogenic microorganisms [26], weed management [27] and treatment of various human diseases [4]. The phenomenon of allelopathy by aromatic plants has been understood for several years [11]. Growth and development of a number of plants were shown to be inhibited by other species, which released inhibitory substances [28]. The present study showed that oxygen-containing phenolic monoterpenes were found to be the major constituents in the essential oil of the two thyme species, followed by moderate amounts of the monoterpenic alcohols while the monoterpenic hydrocarbons came up to the third level. Wild thyme oil consists mainly of oxygen-containing phenolic monoterpenes (68.33%), included carvacrol and thymol caused inhibition for the germination and reduction for hypocotyl and radicle lengths of *L. sativum* and *R. sativus*, seeds at 10^4 mg ml^{-1} oil concentration level. Similarly, the other germination growth parameters (T50, SGI, MGT and GE) were also affected differentially among the two test species. However, at the maximum oil concentration (10^4) cultivated thyme oil, caused less germination inhibition percentage and reduction for hypocotyl and radicle lengths in the two test species compared to that achieved by wild thyme oil. Arminante *et al.* [29] reported that the aerial parts of fresh rosemary and thyme yielded 0.67% and 0.33% essential oil on a fresh weight basis, respectively. Germination was less sensitive than seedling growth. Thyme oil when added at a quantity of 20μl provoked a strong inhibitory effect on the seedling growth even rising to over 75%, while that of rosemary at the same volume was less phytotoxic. Furthermore, De-Feo *et al.* [14] revealed that substances volatilized from the aerial parts of rue, *Ruta graveolens* L. (Rutaceae), exhibited more marked inhibitory effects on radish seeds in darkness, while the pure compounds seem to be inhibitorier in light. The more active compounds seem to affect slightly more the radicle growth.

The results of the present study may be explained on the bases of the chemical composition of the two thyme oils. Oil samples dominated mainly by oxygenated monoterpenes (*T. capitatus*) were significantly more active (67 and 35% of germination inhibition). The allelopathic activity of monoterpenes is thoroughly documented [30]. Amongst the active monoterpenes, there are a variety of oxygenated compounds especially nerol, citronellol, geraniol, linalool, terpinen-4-ol, α-terpineol, borneol, carvone, fenchone, pulegone, camphor, 1, 8-cineol, 1, 4-cineol, carvacrol and ocimenone [15]. It appears that the allelopathic activity showed by the oil of the two thyme species in the present study was due to oxygenated and hydrocarbon monoterpenes content. Although it is not yet possible to attribute such activities to any specific constituent of these oils, it is relevant that major components, or combination of components, from the oil of wild and garden thyme are monoterpenes derived from α-pinene, p-cymene, Para-menthene-1, 1, 8-cineol, borneol, thymol and carvacrol.The volatile phytotoxic substances such as oxygenated phenolic monoterpenes, in different plant organs inhibit germination and growth of many different plant species [31, 32]. For example, volatile compounds such as the terpenoids of *Descurainia sophia* exhibited potent allelopathic effects on eight wheat cultivars at concentrations well below their aqueous solubility [33]. Furthermore, Barbosa *et al.* [34] indicated that, both essential oils rich in sesquiterpenes and that rich in monoterpenes of *Schinus terebinthifolius* showed significant radicle growth inhibition for *Lactuca sativa* and *Cucumis sativus* and significant inhibition of germination of *Lactuca sativa.* The same compounds that presented allelopathic activity as reported in the literature [35].

In conclusion, the reported allelopathic activity observed for the two thyme oils can in part, be explained by the essential oil produced in their shoots. Although, the essential oils affected the growth and development of the two test species, many significant questions remain to be answered before the alleged allelopathic effects of the two thyme species can be confirmed or refuted.

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