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Comparative Study on Composition and Allelopathic Effect of Volatile Oils Extracted from Two *Thymus* Species of the Gebel Akhder in Libya

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***Abstract*----**The present investigation aimed to study the state of the art of the chemical composition of the essential oil extracted from wild (*Thymus capitatus*; TC) and garden thyme (*Thymus vulgaris*; TV) to evaluate the possibility of interrelationship between oil characteristics and allelopathic potentiality using growth bioassays of some test species (*Citrullus colocynthis L.*, *Lepidium sativum L.* and *Trigonella foenum-graecum L.* The essential oils of TC and TV were obtained in yield of about 2.3 and 1.2 % (v/w), on dry weight basis, respectively. The compounds were represented by percentages of about 94.26 and 79.75% respectively. Generally, the oil was grouped into four major classes: hydrocarbon monoterpenes (HM); oxygenated monoterpenes (OM); monoterpene esters (ME) and monoterpene alcohols (MA).

The allelopathic effects of thyme essential oil were tested in vitro on germination percentage (GP) of three different recipient (test) species in oil concentrations from 1 to 20 mg/l in a dose-dependent manner and the germination parameter (GP) of the three bioassay species were markedly decreased in progressing from the control to the maximum oil concentration at 20 mg/l.

The arrangement of the effect of the experimental oils was summarized in the following order TC >TV. It is worth mentioning that the wild growing species; C. colocynthis was more resistant to the thyme oil effect followed by L. sativum then T. foenum-graecum.

***Keywords*----**The allelopathic effects of thyme essential oil (*Thymus capitatus*; TC) and (*Thymus vulgaris*; TV)

I. Introduction

THE genus Thymus L. (thyme) 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 [4].

Thymus capitatus L. (wild thyme) and *Thymus vulgaris L.* (Garden thyme) are two common widely distributed thyme species recorded in Libya. *Thymus capitatus* is a densely glandular-dotted dwarf shrub with small entire leaves, and flowers arranged in terminal heads. It is mostly common in Libya in rocky habitats.

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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%) [20]. Thymol, an antiseptic, is the main active ingredient in different mouthwashes [17].

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 [`13]. Most chemicals that have been identified in allelopathic interactions have been identified as either terpenes or phenolic compounds [8**]**. 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. [16], *Ruta*, *Rosmarinus officinalis L.*, *Thymus vulgaris L. Satureja Montana L.* [2] and *Conyza albida Willd.* [18].

II. Materials And Methods

Plant Materials

The shoots of Thymus capitatus (L.) Hoffmanns. & Link. (wild thyme) were collected during summer 2012 from three rocky ridge habitats distributed on the north eastern al Bayda . As well, the shoots of Thymus vulgaris L. (garden thyme) were collected from four cultivated fields at El- fatieh. Plants were identified and authenticated according to [3].

Extraction of the Essential Oils

The essential oil was extracted from the dried shoots of samples of the two target species (100 g) by hydrodistillation method for three hours using Clevenger-type apparatus. The extracted oils were dehydrated over anhydrous sodium sulphate and the content was determined in four replicates and the mean values were calculated [10**]**.

Gas chromatography-mass spectrometry (GC-MS)

The collected oils were diluted in diethyl ether (20 ml in 1 ml) and analyzed by Gas Chromatography/Mass Spectroscopy (GC/MS) 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.

Groups	Compound	TC (%)	TV (%)
Monoterpene hydrocarbons	p-Cymene	3.21	2.63
	α-Terpinene	2.98	2.81
	α-pinene	3.31	2.33
	myrcene	0.98	0.80
	Camphene	0.44	0.30
	Sabiene	4.35	2.10
	Limonene	0.94	0.60
	α-thujene	0.71	0.45
Oxygenated phenoli	Thymol	14.20	10.74
	Carvacrol	51.18	47.23
Monoterp	1,8-cineol	2.64	2.54
Monoterpene alcohols	1,3-Octadiene	0.54	0.30
	1,7-octadiene	0.26	0.1
	Borneol	5.30	4.92
Other compounds	Para-menthene-1	2.90	1.8
	Para-menthene-3	0.32	0.10
Total		94.26	79.75

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 ºC, 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 indices and the previously published data.

Germination and Growth Bioassays

Bioassays were carried out with seeds of *Citrullus colocynthis (L.)* Schard. (*colocynth, Cucurbitaceae*), *Lepidium sativum L.* (garden cress, Brassicaceae) and *Trigonella foenum-graecum* L. (fenugreek, Fabaceae) (Anti-diabetic plant species). These species were considered as test or recipient species (bioassay materials) in the current study. The three species are often grown or cultivated along the irrigated areas in the northern western Mediterranean region where thyme are widespread. Seeds of colocynth, garden cress and fenugreek were purchased from El-Shefaa Center for Herbal Medicine.

Results Chemical composition of the essential oils extracted from the dry shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* Essential oils solutions previously extracted from the dry shoots othe two thyme species were prepared at concentrations of 1, 5, 10 and 20 mg/l in dichloromethane. Assays were conducted in 9-cm diameter glass Petri dishes lined with one sheet of Whatman No. 1 filter paper and to be sealed with Parafilm. Before sowing the seeds of *Citrullus colocynthis*, *Lepidium sativum* and *Trigonella foenum-graecum* were treated with H2SO4 (96%) for 2 minuets then, rinsed four times with distilled water. To each dish, 10 ml of each solution was added and the solvent was evaporated before addition of 10 ml of water followed by 20 seeds of one of the test species with four replicates. Assays were carried out to investigate the potential allelopathic effects of the five mentioned volatile oils on germination percentage (GP) of the recipient species. Assays were achieved at 25 ºC under artificial fluorescent light (8 x 40 W) in an incubator for 3 days; after germination. Each bioassay was replicated four times in a complete randomized design. Data representing germination percentage (GP), was recorded after three days.

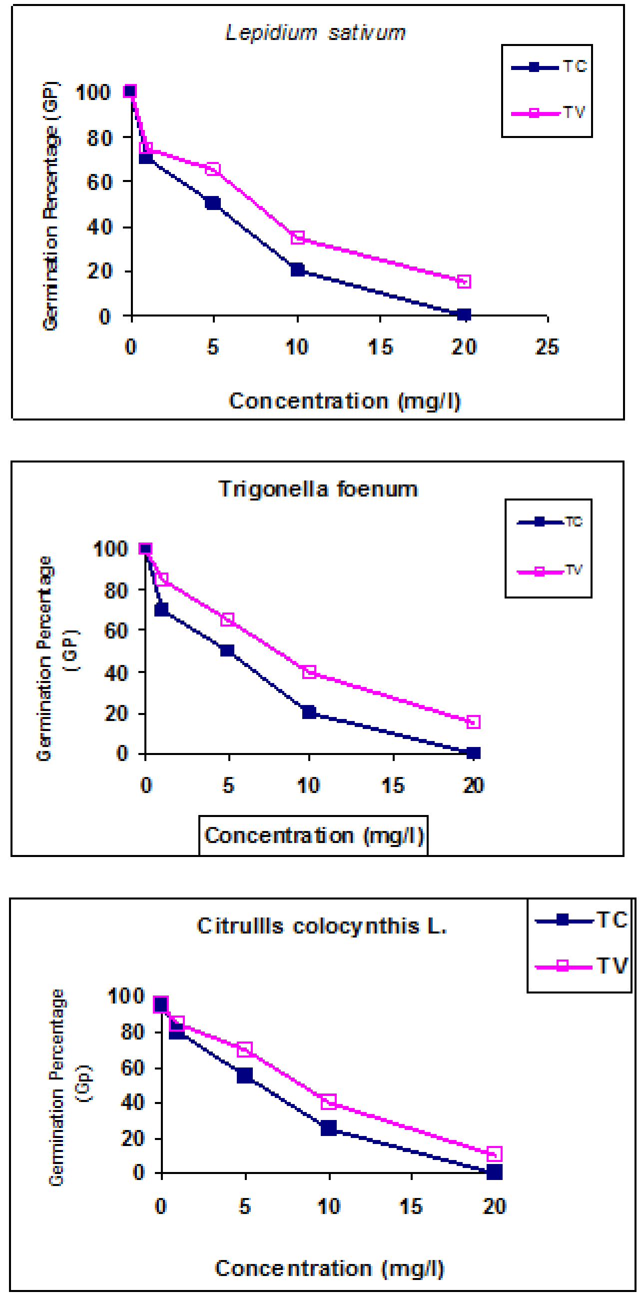

Fig. 1 Allelopathic effect of different concentrations (mg/l) of oil extracted from the dry shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* (TV) on germination percentage (GP) of recipient species.

Germination Bioassays

Bioassays were carried out to test the effect of the essential oils extracted from the shoots of *Thymus capitatus* and *Thymus vulgaris* (target species) upon three different recipient (test) species; *Citrullus colocynthis* (L.) Schard. (colocynth, Cucurbitaceae), *Lepidium sativum* L. (garden cress, Brassicaceae) and *Trigonella foenum-graecum* L. (fenugreek, Fabaceae).

Biological activity of thyme essential oils on some germination and growth parameters of recipient species.

Firstly ***Citrullus colocynthis***. The germination percentage (GP) was notably decreasing with increasing the concentrations of the thyme oils. The percentage decreased from 95% at the control to 25 and 45% at 10 mg/l concentration for *T. capitatus* and *T. vulgaris*, respectively after three days. It is worth to mention that the germination of the seeds of the test species was completely inhibited at the maximum applied concentration (20 mg/l). Secondary ***Lepidium sativum*** the germination percentage (GP) was remarkably decreasing with increasing the concentrations applied of the essential. The percentage decreased from 100% at the control to 20 and 35% at 10 mg/l concentration for *T. capitatus* and *T. vulgaris*, respectively after three days.

Finally ***Trigonella foenum- graecum*** the germination percentage (GP) was notably decreasing with increasing the concentrations of the essential oils. The percentage decreased from 100% at the control to 30 and 40% at 10 mg/l concentration for *T. capitatus* and *T. vulgaris*, respectively after three days. It is worth to mention that the germination of the seeds of the test species was completely inhibited at the maximum concentration (20 mg/l).

III. Discussion

1. Chemical Composition of Thyme Essential Oi

In the present study the hydrodistillation of the dried shoots of *Thymus capitatus* (TC) and *Thymus vulgaris* (TV) gave pale yellow essential oils (yields 2.3 and 1.2% v/w, respectively) based on dry weight basis. The GC-MS analysis of the essential oil of the two thyme species led to the identification of 16 different components, representing 94.26 and 79.75% of total oil constituents, respectively. The essential oil of the two thyme species contains a complex mixture consisting mainly monoterpenes hydrocarbons (16.92 and 12.02%), oxygenated phenolic monoterpenes (65.38 and 57.97%), monoterpene esters (2.64 and 2.54%), monoterpenes alcohols (6.10 and 5.32%), and other compounds (3.22 and 1.9%) respectively. The major oil compounds recognized in the essential oil of *T. capitatus* and *T. vulgaris* are α-thujene (0.71 & 0.45), α-pinene (3.31& 2.33), myrcene (0.98 & 0.80), α- terpinene (2.98 & 2.81), p-cymene (4.19 & 3.63), sabiene (4.35 & 2.1), Para-menthene-1 (2.9 & 1.8), limonene (0.94 & 0.60), 1,8-cineol (2.64 & 2.54), thymol (14.20 & 10.74) and carvacrol (51.13 & 47.23), respectively. The two major compounds identified in the present study were thymol and carvacrol which carry economic importance in medicinal plant use and food industry [9**]**. Thymol also known as **2**-isopropyl-5-methylphenol is a natural monoterpenes phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with Carvacrol, found in oil of thyme, and extracted from *Thymus capitatus and Thymus vulgaris* and various other kinds of plants as a white crystalline substance of a pleasant aromatic odor and strong antiseptic properties [15]**.** Carvacrol, or cymophenol, $C_6H_3CH_3(OH)(C_3H_7)$, is a monoterpenoid phenol. It has a characteristic pungent, warm odor of oregano and a *pizza-like* taste [19]. Carvacrol is present in the essential oil of *Origanum vulgare*, oil of thyme, oil obtained from pepperwort, and wild bergamot. The essential oil of thyme subspecies contains between 5% and 75% of carvacrol, while *Satureja* (savory) subspecies have content between 1% and 45%. The Origanum species majorana and Dittany of Crete are rich in carvacrol, 50% resp. 60-80% [5**].**

2. Allelopathic Effects of Thyme Essential Oil

A plant may interfere with the growth of neighboring plant through competition and/or allelopathy. Allelopathy is an interference mechanism in which living or dead plant parts release allelochemicals exerting a negative or positive effect on surrounding plants and microorganism, and thus can play an important role in natural and managed ecosystems [6]**.** Functionally, plants have evolved several strategies to interact with other organisms, for self defense, sexual attraction, symbiosis, and development [7]**.** The production, accumulation and release of secondary metabolites, which inhibit and/or stimulate germination and development of other plants, is an important mechanism in the interactions between plants. Aromatic plants, known to be rich in active principles, **)** can play an important role in plant-plant interactions and constitute a primary source of potential allelochemics [1] **.**

In the current study, to evaluate the thyme essential oil's possible allelopathic effects, oil concentrations from 1 to 20 mg/l in a dose-dependent manner were tested *in vitro* on seeds germination percentage (GP) of three different recipient (test) species; *Citrullus colocynthis* (L.) Schard. (colocynth, Cucurbitaceae), *Lepidium sativum* L. (garden cress, Brassicaceae) and *Trigonella foenum-graecum* L. (fenugreek, Fabaceae) seeds.

The germination percentage (GP) of the three bioassayed species (*Citrullus colocynthis*, *Lepidium sativum* and *Trigonella foenum-graecum*) were decreased markedly in progressing from the control to the maximum applied oil concentration at 20 mg/l and the reduction percentage was prominent for *T. capitatus* and *T. vulgaris*,the reduction effect was summarized in the following order TC >TV. It is noteworthy that the wild growing species, *C. colocynthis* was more resistant to the thyme oil effect followed by *L. sativum* then *T. foenum-graecum* for most of the estimated parameter. Our data agree with the literature on inhibitory activity exerted by essential oils on seed germination. respectively. A little difference was noticed in the chemical composition between the found allelochemicals are phenolic compounds and terpenoids**.** 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 [12].

IV. Conclusion

Evaluation of biological potentialities of a North Africa plant; *T. capitatus and T. vulgaris* was our objective in this work. Results revealed a very toxic effect of the essential oil on seed germination and growth. So, our results are encouraging and show the interest of extracts of *T. capitatus and T. vulgaris*. This species presents an inheritance thus to be preserved and develop in order to apply in therapeutic and agro-alimentary fields.

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