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Allelopathic Effects on Seeds Germination of Lactuca Sativa L. Seeds and Antibacterial Activity of Thymus Capitatus Essential Oil from Zintan-Libya flora

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Abstract

The present study was amid to evaluate the essential oil composition of *Thymus capitatus* and its allelopathy effects on seeds germination of *Lactuca sativa* L., based on germination assay, also antibacterial activity was tested against to bacteria species (Salmonella gallinarum and E. coli) using the disc diffusion method. The oil analysis by GC-MS, showed that the main class was oxygenated monoterpenes with percentage (87.61 %), while hydrocarpons monterpend represented by (10.46 %) of the total oil, and the dominated compounds were carvacrol (68.19 %), thymol (12.29 %) and p-cymene (3.25 %) respectively. The allelopathy effect based on germination assay, where we used three essential oil concentration (0.5 µl/ml, 1 µl/ml, 2 µl/ml) showed that the activity increase with the increase of the oil concentration and the inhibition percent (IP) reached 96.66 % and germination percentage (GP) 3.4 % at the maximum concentration (2µl/ml) compared with control which was only water IP 0 % and GP (100%). In the case of antimicrobial activity the oil showed activity better than positive control (Gentamycine) against the two bacteria species and diameter of the clear zone around the discs were (35 mm, 32mm, 15 mm) for *S. gallinarum*, *E. coli* and positive control respectively.

Keywords: Allelopathy; antimicrobial activity; essential oils; Thymus capitatus.

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1. Introduction

Farmer and researcher are continuously trying to find the most effective way to management the weeds in farming. The use of chemicals to control weeds problems lead to make herbicides more resistance [1].

Hence, the effective method to manage the weeds is to reduce the control costs.

Allelopahty is a phenomenon, which has a great deal with weeds control. Plants release chemicals in the environment, these chemicals could reduce or inhibition the germination of seeds or reduce the growth of other species and influence the natural population [2].

Because of the increase of herbicide resistance, allelopathy could be used as alternative method to control the weeds [3]. The plants extraction such as essential oil and different extracts has been reported to contain chemical compounds which have the ability to inhibit the seeds germination depending on their chemical profile [3].

This genus of Thymus is considered as one of the most genus which is rich in essential oils, this genus is represented by only two species In Libya, - Thymus algeriensis Boiss. et Reut. and Th. capitatus Hoffms. et Link. Which known under the common name "Zaatar"[4]. These species are commonly used fresh or dry as spicy herbs, for medicine purpose against illnesses of the digestive tube, to treat respiratory system disorders and antiabortion [5:6].

Th. capitatus is a low shrub 20-60 cm; stems much branched, compact; old branches stiff, spine scent, whitish; leaves 3-8 x 0.5-1 mm, linear, keeled and boat-shaped, gland-dotted, ciliate at the base, sessile; flowers in ovoid heads 0.6-1.2 cm; bracteoles 5-6 x 1.5 mm, ovate, the margins ciliate; calyx 4-5 mm, bilabiate, 13- veined; upper lip with 3 triangular teeth, the lower 2-fid, with subulate teeth; corolla 0.8-1 cm, bilabiate, purplish-mavue, with red glandular dots outside, exserted; stamens exerted; style branches equal [7]. Also it has been reported to have biological activities such as antimicrobial and antitumor activities [8], also, the allelopathic effect have been reported.

Since, the activity of the essential oils is related to the chemical composition of the oil. The Amis of this study was to evaluating the allelopathic effects of *Th. capitauts*, essential oil, which was collected from Zinatn- Libya based on seeds germination assay, to determined the minimum concentration of the oil, which can inhibit the germination of seed, Also, to evaluate the antibacterial properties in vitro. The seeds used in this study were belonged to Lactuca sativa, which used to give an indicator to this activity.

2. Material and Methods

2.1. Plant collection

The wild growing sample of investigated plant were collected during the flowering stage from Zintan (Libya), which located on the top of Western mountain (Aljabel Algarbi) at altitude about 700 m above sea level [4].

The plant were identified by Dr. A. Giweli, Faculty of Science, Al-Gabel Al- Garbi University Libya, and later confirmed by (Dr. R. Almahdy), Faculty of Science, Al-Gabel Al- Garbi University Zintan –Libya. The sample was dried in shadow at room temperature for 10 days.

2.2. Essential oils isolation

Most reported studies which deal with the essential oil of plants have made use of hydro-distillation in Clevenger-type apparatus [9:10]. In this study we have isolated the essential oil by using hydrodistillation method. Amount of (100g) of air-dried aerial parts of plant samples derived from wooden parts, were submitted to hydrodistillation, using Clevenger-type apparatus for 2 h, according to the standard procedure reported earlier in the [11].

2.3. **Gas chromatography and gas chromatography - mass spectrometry (GC-MS) analysis**

Qualitative and quantitative analyses of the oils were performed using GC and GC-MS. The GC analysis of the oil was carried out on a GC HP-5890 II apparatus, equipped with split-splitless injector, attached to HP-5 column (25 m x 0.32 mm, 0.52 µm film thickness) and fitted to FID. Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-240°C (at rate of 4°/min). The same analytical conditions were employed for GC-MS analysis, where HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-400. Identification of the individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with those held in Wiley275 library of mass spectra. Confirmation was performed using AMDIS software and literature [12]. For the purpose of quantitative analysis area percent obtained by FID were used as a base.

2.4. Antibacterial assay

The antibacterial activity of the oil was made by using disc diffusion method according to [13], with some modifications against two bacteria species obtained from Tripoli Veterinary Center (*S. gallinarum* and *E. coli*).

The bacteria were inoculated in nutrient broth for 24 hours on 37 ºC. The sensitivity test was performed by inoculating the bacteria from concentrated broth or from a 10^5 dilution with PBS. 100 µl of the bacterium is swabbed on Mueller Hinton Agar, then separated on Petri dish by cotton swap and left for 15 minutes.10 µl of essential oil (Th. capitatus) were put on a small disc of Whatman filter paper No1(6 mm) and placed on Agar, Gentmycine was used as a positive control to evaluate the sensitivity of each microorganisms. After 24 hours of incubation at 37 ºC, the diameter of a clear zone around the discs were used and expressed in millimeters as its antibacterial activity. The test was run in duplicate.

2.5. Allelopathic bioassay

A bioassay based on germination was used to study the possible allelopathic effects of *Th. capiataus* essential oil on seeds of Lactuca sativa L. The seeds were surface- sterilized in 95% ethanol for 15 s and then washed 3 times in distilled water. Thirty seeds sown in Petri dishes (90 mm diameter), containing two layers of Whatman filter paper No1, impregnated with 3 ml of distilled water (control) or 3 ml [14] of different doses of the essential oil (0.5, 1, 2 µl/ml). 50 µl of the oil were dissolved in 50 µl ethanol (1:1) then distilled water was added to get a stock solution with concentration 10 µl/ ml and diluted to the properate concentration. Controls performed with this mixture alone showed no appreciable differences with control. The Petri dishes of all treatment incubated at 20±1ºC. Seeds were observed daily and considered germinated when the radical was approximately 1 mm long or more. Also the only water added when necessary. All experimental were carried in replicate. The inhibitory or germination percent was calculated using the following equations given by Chung et al. [15]. $GP=100*\frac{n}{N}$ and $IP = \frac{(Cn-Tn)}{Cn} * 100$

Where: GP = germination percentage, n = number of seeds germinated, N = total number of seeds, IP = inhibitory percent, Cnl = number of seeds germinated in control, Tn = number of seeds germinated in every treatmen..

3. Results

3.1. Essential oil composition

The GC and GC-MS analysis of the essential oil of Th. capitatus is reported in **Table 1.** Twenty nine compounds were identified representing 99.71% of the total oil. The oil yield obtained was 4.97 % (w/v). The monoterpenes was the most abundant in the oil with percentage (98.06%) of the total oil. The oxygenated monoterpenes class being the most representative (87.60%), followed by monoterpene hydrocarbons 10.46% and sesquiterpenes reached (1.06%) of the total oils. The result of oil analysis showed that carvacrol (68.19%) was main compound, followed by thymol, p-cymene, y-terpene, p-cymene-7-ol, borneol, linalool and β-caryophyllene (12.29%, 3.25%, 3.09%, 2.83%, 1.58%, 1.21% and 1.02% respectively). Other compounds were represented in a trace amount less than 1% of the total oil.

3.2. Antibacterial activity

The findings of antibacterial assay were reported in **Table 2, Figure 1.** The essential oil of Th. capitatus, the zone of in inhibition which caused by the oil was 35 mm against S. gallinarum and 32mm for E. coli , respectively, while Gentamycine made inhibition zone with 15 mm.

3.3. Alellopathy assay

The results of allopathic effect of *Th. capitatus* essential based on germination assay were reported in **Table 3, Figure 2.** It can be seen that the PG decrease with the increase of essential oil concentration, the PG of the different concentration were (100%, 96.7%, 95%, 10%, and 3.3%) for w, wa, 0.5, 1, 2 µl/ ml respectively. In the same, the IP were decrease with the increase of the oil concentration (0%, 3.3%, 5%, 90%, and 96.7%) for (w, wa, 0.5, 1 and 2 µl/ ml) respectively.

Table 1: The chemical composition of Thymus capitatus essential oil from Libyan flora

No	**Constituents**	**KIE**	**KIL**	**% ID**
1	α-thujene	917.9	924	0.67
2	α-pinene	923.5	932	0.49
3	camphene	937.8	946	0.33
4	sabinene	961.0	969	0.12
5	β-pinene	966.1	974	0.36
6	β-myrcene	983.4	988	0.92
7	α-phellandrene	995.6	1002	0.12
8	δ^3-carene	1001.4	1008	0.05
9	α-terpinene	1007.9	1014	0.75
10	p-cymene	1016.0	1020	3.25
11	β-phellandrene	1019.5	1025	0.28
12	1,8-cineole	1022.5	1026	0.08
13	trans-β-ocimene	1040.4	1044	0.03
14	γ-terpinene	1049.7	1054	3.09
15	cis-sabinene hydrate	1059.8	1058	0.29
16	α-terpinolene	1078.9	1086	0.10
17	linalool	1093.3	1095	1.21
18	camphor	1135.1	1141	0.17
29	borneol	1157.2	1165	1.58
20	terpinene-4-ol	1168.9	1174	0.65
21	cis-dihydro carvone	1183.7	1191	0.09
22	trans-dihydro carvone	1195.0	1200	0.12
23	p-cymen-7-ol	1283.2	1287	2.83
24	thymol	1289.4	1289	12.29
25	carvacrol	1303.9	1298	68.19
26	thymol acetate	1338.8	1349	0.08
27	β-caryophyllene	1406.9	1417	1.02
28	α-humulene	1441.5	1452	0.05
29	caryophyllene oxide	1570.1	1582	0.51
				%ID
Sum of contents				99.71
Number of constituents				29
Monoterpene hydrocarbons				10.46
Oxygenated monoterpene				87.60
Sesquiterpene hydrocarbons				1.06
Oxygenated hydrocarbons				0.51
other				0.08

Table 2: The inhibition zone of essential oil and control in (mm)

Bacterai spp	S. gallinarum	E. coli	Gentamycine
Th. capitatus oil	35	32	15

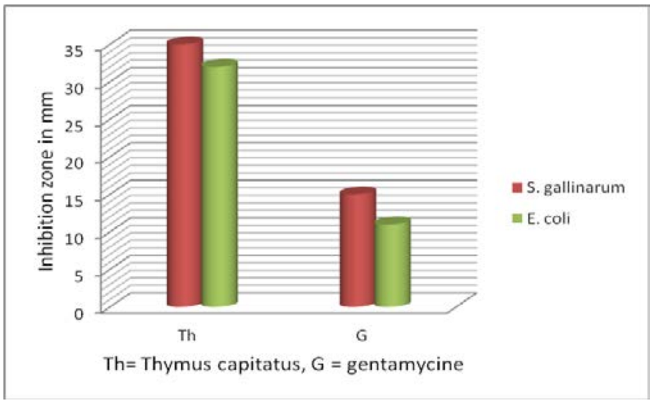

Figure 1: value of inhibition zone of Th. capitatus essential oil

Table 3: The inhibition percentage (IP) of oil on seeds

Concentrations of essential oils	Control (w) 0 µl/ ml	Wa 0 µl/ ml	0.5 µl/ ml	1 µl/ ml	2 µl/ ml
IP	0	3.3	5	90	96.7

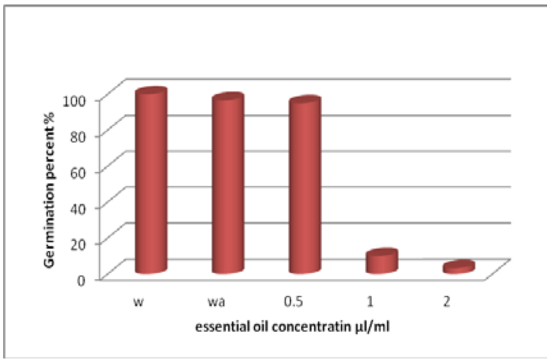

Figure 2: The germination percentage (GP), W= distilled water, wa= water & ethanol, 0.5 = essential oil concentration 0.5 µl/ ml, 1= essential oil concentration 1 µl/ ml and 2 = essential oil concentration 2 µl/ ml

4. Discussion

4.1. Chemical composition

Several reported have been interested in the biologically active compounds extracted from plant materials for food preservation [16], weed manage management [17]. The phenomenon of alellopathy by aromatic plans has been not understood for many years [2].

The seeds germination and plants growth of a number of species could be inhibited by other plant species, which produce inhibitory substances [17]. In our study the oil composition of the Th. capitatus essential oil, showed that the monoterpenes was the most abundant representing by (98.06%) of the total oil. Among the monoterpenes the class oxygenated monoterpenes was the dominated class (87.6%), followed by hydrocarbon-monoterpenes (10,46 %).

The main compounds of the oil were carvacrol (87.6%) , followed thymol, p-cymene, y-terpene, p-cymene-7-ol, borneol, linalool and β-caryophyllene (12.29%, 3.25%, 3.09%, 2.83%, 1.58%, 1.21% and 1.02% respectively), which mean that essential oil of Th. capiataus collected from Zinan is carvacrol, thymol chemo-type. These results are in similar with previous reported by [8, 18]. However, our results were in contrast with Skoula and Grayer [19], who reported that the oil of Th. capitatus was thymol chemo-type.

4.2. Antibacterial activity

According to the results, which have been reported in **Table 2, Figure 1**, it can be seen that the two species of bacteria were sensitive to the essential oil. The inhibition zones were 35 mm and 32 mm for S. gallinarum. and E. coli respectively compared with 15 mm and 11 mm for control, it is obvious that the oil showed antibacterial activity two times better than Gentamycine. Also, S. gallinarum was more sensitive to oil Th. Capitatus oil than E. coli.

Comparing the previous data with the chemical composition of the oil, it becomes evident that there is a relationship between the high activity of the Thymus type oils and the presence of phenolic components, such as thymol and carvacrol. The high antimicrobial activity of this essential oil could be explained by the high percentage of phenolic components. It seems possible that phenolic components may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α- and β-glucanases of the fungus [20]. Consequently, the high content of phenol components may account for the high antibacterial activity of the oils [21].

According to our results it can be seen that essential oil of the investigated species as well as individual phenolic monoterpenes carvacrol and thymol have very high antimicrobial activities, even higher than the commercial antibiotic Gentamycine. These results could be related to the high percentage of phenolic compounds, such as carvacrol and thymol [22:23:24]. Furthermore, it has been reported that the antifungal activity of Thymus pallescens from Oued Rhiou or El-Asnam regions in Algeria is attributed to their high content in thymol and carvacrol [25]. Also, the antibacterial activity of Th. algeriensis collected fron Zintan was related to phenloic compounds [26]. In fact, it is difficult to attribute the antimicrobial activity of Th. capitatus essential oil, characterized by a complex mixture, to a single or particular constituent. In fact, some studies have concluded that whole essential oils have a greater antibacterial activity than a mixture of all the major components [27].

4.3. Alellopathy effects of the oil

The results of alellopathic effects of Th. capitatus essential oil on seeds germination of L. sative showed in **Table 3**, **Figure 2,** indicated that the inhibition of seeds germination increase with the increase of the oil concentration. The PG values were significantly reduced at oil concentration 1 and 2 µl/ ml (10% and 3.3%) and

IP (90% and 96.7%) respectively compared with control which was only water PG (100%) and IP (0%).

The effects of essential oil on seeds germination could be attributed to oil composition spicily the monoterepens [28]. Also, among the monoterepens the classes (oxygenated- monoterepens and hydrocarbon-monoterepens), which contain carvacrol and thymol could be another reason for this activity [29], which were the most abundant compounds in the oil and they were represented together 98.06%.

However, , it cannot attribute these activity to one or two specific compounds, but the mixture of main and minor compounds are responsible for the activity [30:31].

5. Conclusion

The reported allelopathic activity in this study for Th. capiataus essential oil, could be attribute to high presence of oxygenated monoterpenes group in the oil, also the same group could be responsible for antibacterial activity. More work should be done to confirm these results by using different plant seeds. However, the Th. capiataus essential oil may have potential as an allelopathic and an antimicrobial agent in many applications. The fact that essential oil is a natural product makes it attractive, and further research to elucidate its mode of action is necessary.

6. Recommendations

- More works should be done to confirm these results.

- Same work should be done in the field to evaluate the action of essential oil of *Thymus capitatus* on seed germination of weeds in real environment.

- Different seeds of weeds should be tested to confirm the alellopathic effects of the oil.

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