International Journal of Mosquito Research

ISSN: 2348-5906
CODEN: IJMRK2
IJMR 2017; 4(1): 37-42

Received: 15-11-2016
Accepted: 16-12-2016

Nadia Bouguerra
(1). Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Algeria
(2). Laboratory of Applied Animal Biology, Faculty of Sciences, Department of Biology University Badji Mokhtar of Annaba, Annaba, Algeria

Fouzia Tine Djebbar
(1). Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Algeria
(2). Laboratory of Applied Animal Biology, Faculty of Sciences, Department of Biology University Badji Mokhtar of Annaba, Annaba, Algeria

Noureddine Soltani
Laboratory of Applied Animal Biology, Faculty of Sciences, Department of Biology University Badji Mokhtar of Annaba, Annaba, Algeria

Correspondence
Nadia Bouguerra
(1). Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Algeria
(2). Laboratory of Applied Animal Biology, Faculty of Sciences, Department of Biology University Badji Mokhtar of Annaba, Annaba, Algeria

Algerian Thymus vulgaris essential oil: chemical composition and larvicidal activity against the mosquito *Culex pipiens*

Nadia Bouguerra, Fouzia Tine Djebbar and Noureddine Soltani

Abstract
The objective of the present study was to assess the chemical composition of the essential oil extracted from *Thymus vulgaris* leaves and their larvicidal activity against fourth instar larvae of *Culex pipiens*, the most abundant mosquito species in Tebessa area (North east Algeria)**.** The essential oil yield of the plant was 1.58 %. The GC/MS analyzes revealed eighteen compounds in essential oil of *T. vulgaris*. The major chemical component identified was linalool (82.88 %). The dominant monoterpene fraction (98.48 %), contains oxygenated monoterpenes (94.31%) and monoterpene hydrocarbons (4.17%). Sesquiterpenes and secondary alcohol represents only 2.05% and 0.24%, respectively. In a second series of experiments, the larvicidal activities of *T. vulgaris* essential oil was evaluated against fourth instar of *Cx pipiens* at different concentrations (25, 50, 100, 150 and 200 ppm). The LC50 values obtained at 24, 48 and 72 hours after treatment were 72.04, 68.61, 62.12ppm respectively, and the LC90 values were 207.01, 190.54 and 169.82, respectively. The results obtained show that this plant material exhibited significant activity and could be considered as potent natural larvicidal agent against *Cx pipiens*.

Keywords: *Culex pipiens,* Essential oils, GC/MS, *Thymus vulgaris,* Toxicity.

1. Introduction

Several mosquito (Diptera: Culicidae) species are primary vectors for many dreadful and fatal diseases like dengue, Japanese encephalitis, filariasis, yellow fever, malaria and chikungunya, they can transmit a variety of pathogens that infect humans and other animals than any other group of arthropods [1-3]. The proliferation of these diseases is due to the increasing resistance of mosquitoes to the used conventional insecticides [4]. The extensive and indiscriminate application of these insecticides in mosquito controls caused diverse problems such as environmental pollution, insecticide resistance, and harmful effects to non target organisms [5, 6]. Consequently, alternatives to synthetic insecticides has been searched such as botanicals, which are effective, biodegradable, ecofriendly, and inexpensive [7]. Approximately, 2000 plant species have been known to produce secondary metabolites of value in biological pest control programs and only 344 plant species showed insecticidal activity against mosquitoes [8]. Many studies have looked at the possibility of using plant extracts as growth inhibitors and larvicides against mosquitoes [8-13]. The genus *Thymus* (Lamiaceae) is an aromatic plant that includes about 215 species particularly in the Mediterranean area, including eleven (11) species in Algeria [14]. Some studies have shown that *Thymus* species have strong antibacterial, spasmolytic, antiparasitic, antifungal, antioxidant and antiviral activities [15]. In addition, it has been reported that *Thymus* species have strong insecticidal activity against various insects such as mosquitoes, *Aedes albopictus* [16], *Aedes aegypti* [17], *Culex pipiens* [18-20], *Anopheles labranchiae* [21], and insect pests [22-25]. Recently, Dahchar *et al* [26] have tested the larvicidal potential of aqueous extracts from leaves of three plants (*Ricinus communis* L., *Daphne gnidium* L. and *Thymus vulgaris* L.) against the different larval instars of *Culex pipiens* and *Culiseta longiareolata*, while Dris *et al*. (2017) [27] have determined the chemical composition of the leaf essential oil of *Lavandula dentata* L. cultivated in Tebessa (Algeria) and its activity against fourth instar larvae of *Cs longiareolata* and *Cx pipiens*. In this study conducted under laboratory conditions, in continuation to our previous studies, aimed determination of the chemical composition of *Thymus vulgaris* essential oil by GC/MS. In a second series of experiments, we assessed the efficacy of this essential oil against fourth-instar larvae of *Cx pipiens*, the most abundant mosquito species in Algeria particularly in Tebessa area [28] by determining the lethality parameters.

2. Materials and Methods

2.1 Plant material and extraction of the essential oil

The fresh aerial parts of *Thymus vulgaris* samples were collected January-march 2016 from Blida (North-Algeria) (36°29' N, 2°50' E, Altitude: 229 m). The air-dried aerial parts of the plant were submitted for 3h to steam distillation, using a Clevenger type apparatus. The obtained essential oil was dried with anhydrous sodium sulphate and stored at 4°C in dark vials until tested by GC–MS.

2.2 Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) analysis were performed with an HP Agilent 2890 plus gas chromatograph (GC) equipped with a HP-5MS column (a length of 30 m × internal diameter of0.25 mm, and 0.25 mm film thickness). The column oven temperature was set at 60 ◦C for 8 min, and then increased to 250 ◦C at rate of 2 ◦C/min. The injector and detector temperatures were kept respectively at 250 and 270 ◦C. Carrier gas was helium, the flow through the column was1ml/min, and the split ratio was set to 50:1 with injection of (0.2) µl of oil sample (Table1). The GC/mass spectrometry (MS) analysis was performed with a Quadrupole mass spectrometer that operated at 70V. Constituent's identification was based on comparison of retention times with those of corresponding reference standards using the NIST and WILEY libraries [29, 30]. Percentage compositions of essential oils were calculated according to the area of the chromatographic peaks.

Table 1: General information on GC-MS analysis performed.

Column type	**HP-5MS (5% Phenyl, 95% dimethylpolysiloxane) 30m*0.25mm* 0.25µm**
Injection volume	0.2µl
Injection temperature	250 °C
Interface temperature	270 °C
Mode of injection	Split
Vector gas	Helium

2.3 Mosquito rearing

Culex pipiens eggs and larvae were collected from untreated areas located at Tebessa (Northeast Algeria). Each 20 larvae were kept in pyrex storage jar containing 150 ml of stored tap water and maintained at temperature between 25-27°C. Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight) [31], and water was replaced every three days.

2.4 Treatment and insecticidal activity

Bioassays were conducted as previously described [32]. The essential oil of *T. vulgaris* added to treatment beakers at different final concentrations. Newly ecdysed fourth-instar larvae of *Cx pipiens* was exposed to the different concentrations (25, 50, 100, 150, and 200ppm) for 24h in accord with World Health Organization (WHO) criteria [33, 34]. Essential oil was dissolved in 1 ml ethanol, and then diluted in 150 ml of filtered tap water to obtain the desired final concentrations. The controls were prepared using 1 ml of ethanol in 150 ml of water for positive controls and no additive with negative ones. After the exposure time of 24 h, larvae were removed, washed with untreated water and placed in clean water. The test was carried out with 4 replicates each containing 20 larvae per concentration. Mortality was registered at 24, 48 and 72 hours following treatment. Lethal concentrations (LC50 and LC90), 95% confidence limits (95% CL), and slope of the concentration-mortality were calculated.

2.5 Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as the mean ± standard deviation (SD). The significance between different series was tested using Student's *t* test. All statistical analyses were performed using Prism v 6.01 for Windows (Graph Pad Software Inc., www.graphpadwith a significant level $p \leq 0.05$.

3. Results and Discussion

3.1 Extraction yield

The essential oil isolated by hydrodistillation from the aerial parts of *T. vulgaris* was found to be a pale yellow liquid, obtained in yield of 1,58%. The same yield (1.57%) of *T. vulgaris* was reported by Ozcan and Chalchat [35]. This yield is high compared to that in Morocco with 1% [36, 20], to that obtained from *T. leucospermum* (0.84%) and *T. teucrioides subsp. candilicus* (1.23%) [18] and from the leaves of *T. vulgaris* L. collected from Brazil with an average oil yield of 0.25% [37] and from Romania (1.25%) [38]. Our yield is low from another species: *T. pallescens* with a yield ranging from 2.7 to 6.2% from Sidi-Aissa, Boussaada, Oued Rhiou, Kadiria and El Asnam were 3.2, 2.8, 6.2, 3.3, and 2.7%, respectively; and *T. dreatensis* with a yield of 2.3%. While that this yield is higher compared to *T. algeriensis* from Chrea National Park 0.4-0.7% and from El Asnam 0.5% [39]. Steam distillation of essential oils of aerial parts of *Thymus capitatus* and *Marrubium vulgare* L. collected at North cost of Egypt yielded 0.5% and 0.2%, respectively [2]. This differnce in essential oil of *Thymus* yield of the different regions is normal, since it depends on diverse factors like the species, harvest period, geographical origin, period drying and localization, temperature and extraction technique, etc. [40, 41].

3.2 Chemical composition of essential oil

The GC-MS analyses resulted in the identification of eighteen (18) compounds, representing 100% of the total detected constituent's essential oil. The percentages and the retention time of the identified compound of this essential oil were summarized in table 2. The oxygenated monoterpenes linalool (82.88%), thymol (4.923%), linalyl acetate (2.434%), ascabin (1.741%), camphor (0.802%), α-terpineol (0.341%), eucalyptol (0.217%) and borneol (0.176%) were the main components. Cymene (2.082%) followed by γ-terpinene (0.923%), camphene (0.434%), ß-myrcene (0.230%), α-pinene (0.217%), α-thujene (0.153%), α-terpinene (0.139%) were the major monoterpene hydrocarbons. Sesquiterpene hydrocarbons and oxygenated sesquiterpene were mostly represented by caryophyllenne (1.470%) and caryophyllenne oxide (0.586%). The fraction of non terpenic compounds was represented by secondary alcohols (1-octen-3-ol) (0.245%).

These results are partly in accordance with those reported by Houmani *et al*. [42] for two *T. algeriensis* samples also collected from the same location, Blida (North-Algeria), of which one was linalool-rich (78.80%) and the other thymol-rich (62.7%). Our results differ from those obtained by Dob *et al.* [43] who studied the oil composition of *T. fontanesi* growing wild in Djelfa (South-Algeria), in which thymol (29.3%), *γ*.-terpinene (21.7%), *p*.-cymene (15.9%), and thymol methyl ether (11.4%) as the major components, while a smaller amount of linalool (4.8%) and *β*.-caryophyllene (2.9%). Benayache *et al.* [44] have found thymol (54.1%) followed by p-cymene (15.3%), linalool (5.4%), carvacrol (3.8%), thymoquinone (3.7%), a -pinene (2%), thymol methylether (1.7%) and b-caryophyllene (1.8%) of *T. numidicus*. Chemical composition of the essential oil of *T. vulgaris*, grown in Morocco revealed that thymol content of 41.4%, the dominant monoterpene fraction with 97.35 %, consisting of 46.5 % in the oil form and 50.85% in oxygenated compounds form, hydrocarbons sesquitepéniques represent only a small percentage of 1.7% [20]. In Tunisia, for the cultivated species, linalool was found as chemotype (47.30 %) and linalyl acetate (28.65%), bicyclogermacrene (3.40%), camphor (2.32%) and δ-terpineol (1.47%) were registered by Msaada *et al.* [45] as major compounds. Chemical composition of *T. vulgaris* essential oil from Brazil revealed a predominance of carvacrol (45.5%), α-terpineol (22.9%), and endo-Borneol (14.3%) %) [46]. According to Piccaglia *et al.* [47], six chemotypes has also been reported, geraniol, linalool, *γ*- terpineol, carvacrol, thymol and *trans*-thujan-4-ol/terpinen-4-ol. The content of the essential oil and its composition depend on different factors, environmental and genetic factors, the species, nutritional status of the plants, cultivation conditions such as climate, geographic origin, harvesting time and use of fertilizers [35, 48].

Table 2: Chemical composition of essential oil of *Thymus vulgaris* analyzed by GC-MS.

Nº.	Compound	Retention time	Percentages (%)
Monoterpene			**97.70**
Oxygenated Monoterpene			**93.52**
1	Linalool	21.363	82.88
2	Camphor	23.315	0.80
3	Borneol	25.060	0.17
4	α-terpineol	26.901	0.34
5	Linalyl acetate	31.301	2.43
6	Thymol	34.906	4.92
7	Ascabin	61.938	1.74
8	Eucalyptol	15.170	0.21
Hydrocarbon Monoterpene			4.17
9	α-Thujene	8.780	0.15
10	α-pinene	9.136	0.21
11	Camphene	9.965	0.43
12	ß-myrcene	12.640	0.23
13	α-terpinene	14.259	0.13
14	Cymene	14.871	2.08
15	γ-terpinene	17.209	0.92
Sesquiterpene			2.05
OxygenatedSesquiterpene			0.58
16	Caryophyllene oxide	51.629	0.58
Hydrocarbon Sesquiterpene			1.47
17	Caryophyllene	41.740	1.47
Secondary alcohol			0.24
18	1octen-3ol	12.182	0.24
Total			100%

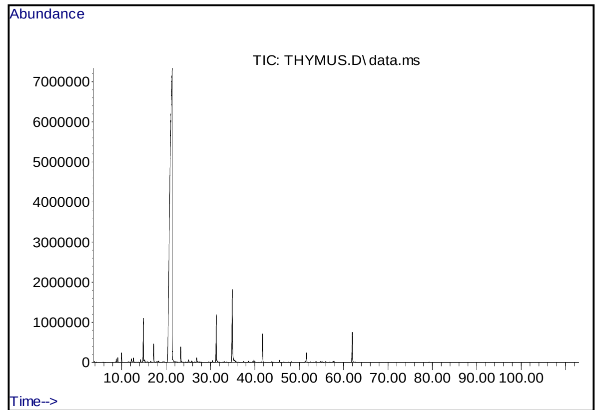

Fig 1: GC-MS chromatogram of *Thymus vulgaris* essential oil (Abundance as function the retention time in min).

3.3 Larvicidal activity

The application of *T. vulgaris* essential oil at various concentrations on fourth instar larvae of *Cx pipiens* exhibited insecticidal activity with dose-response relationship (Figure 2, Tables 3 and 4). The mortality was scored at 24, 48 and 72hours after treatment. The positive controls showed any effect of ethanol against *Cx pipiens*. The lethal concentrations show a variations according to the periods after treatment: 24 (LC50: 72.04 and LC90: 207.01 ppm), 48 (LC50: 68.61and LC90: 190.54 ppm) and 72 hours (LC50: 62.12 and LC90: 169.82 ppm). Our results indicate that *T. vulgaris* essential oils and their active components could be developed as control agents against mosquito larvae. *T. vulgaris* used as aqueous extract or in the form of essential oils was found to present larvicidal activity [49]. The aqueous extracts of the three plants tested, *Ricinus communis* L., *Daphne gnidium* L. and *Thymus vulgaris* L. were found more effective against *Cx pipiens* and the highest activity was found for *T. vulgaris* extracts [26]. Our values are higher to those found with *T. broussonetti* (0.23ppm) and *T. maroccanus* (0.31ppm) against *Cx pipiens* larvae [50], with *Ocimum basilicum* against *An. stephensi* and *Cx quinquefasciatus* (8.29 and 87.68 ppm respectively) [51], and with *Kelussia odoratissima Mozaffarian* against *Culex pipiens* and *Anopheles stephensi* (2.69 and 7.90 ppm) respectively [13]. Similar results were found by Traboulsi *et al.* [12] showing the insecticidal activity of four medicinal plants harvested in Lebanon (*Origanum syriacum* L., *Lavandula stoechas* L., and *Mentha microphylla* K., *Myrtus communis* L.) against *Cx pipiens molestus* (LC50 varying from 16 to 89 mg/L). Sosan *et al.* [52] reported larvicidal activities of essential oils of *Ocimum gratissium, Cymbopogon citrus,* and *Ageratum conyzoides* against *Aedes aegypti* and achieved 100% of mortality at 120, 200, and 300ppm concentrations, respectively. Previous experiments showed that some plant essential oils exhibit larvicidal activity properties such as the metabolites of the marine alga *Caulerpa racemosa* applied to *Cx pipiens* [53], *Tagetes patula* essential oil against three mosquito species *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus* [54], *Origanum syriacum* L*, Lavandula stoechas* L [12] and *Kelussia odoratissima* Mozaffarian applied to *Cx pipiens* [13].

Several factors affect the bioactivity of essential oils including plant species (variety), cultivating conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction [16, 55].

Table 3: Efficacy of essential oil of *Thymus vulgaris* applied on fourth instar larvae of *Cx pipiens*: corrected mortality (m ± SD, n = 4 replicates each containing 20 larvae).

Exposure time (hours)	25ppm	50ppm	100ppm	150ppm	200ppm	P
24	11.67± 2.22	35.00±10.00	58.33± 2.22	83.33± 2,22	98.33 ±2.22	0.0001
48	11.75± 4,33	37.37± 4.91	59.39± 2,92	88.07±2.57	98.33 ±2.22	0.0001
72	10.26±3.33	43.07±4.15	65.53± 1.93	89.56±3.63	100.00± 0.00	0.0001

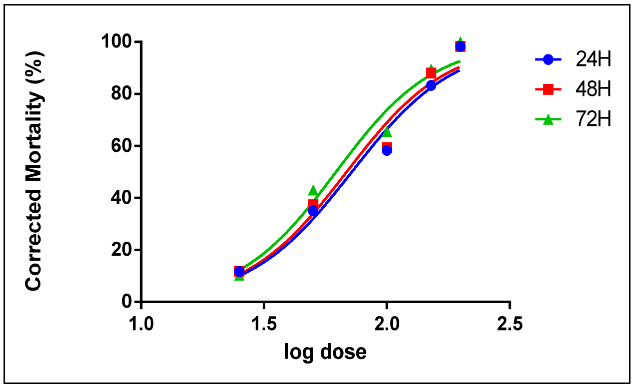

Fig 2: Efficacy of *thymus vulgaris* essential oil against fourth instar larvae of *Cx pipiens.*

Table 4: LC50 and LC90 of *Thymus vulgaris* essential oils against fourth instar larvae of *Cx pipiens*.

Periods	LC50		LC90		Slope	R^2
	ppm	95% FL	ppm	95% FL		
24H	72.04	(52,82 - 98,25)	207.01	(112,71 – 380,18)	2,08	0,96
48H	68.61	(49,40 - 95,30)	190.54	(102,32 – 366,43)	2,11	0,96
72H	62.12	(45,92 - 84,05)	169.82	(94,84 – 306,90)	2,17	0,97

Conclusion

Phytoproducts possess different bioactive components that can be used as general toxicants against various larval stages of mosquitoes [56]. In the present study, it can be concluded that the essential oil of *T. vulgaris* with linalool and thymol as major compounds was found to exhibit potent larvicidal activity against *Cx pipiens* larvae. Further studies on commercial preparation of repellent products and field trials are needed to recommend the development of ecofriendly chemicals from this plant based oil for mosquito control and protection against the bites of haematophagous insects. Moreover, information on the effects of these essential oils on growth and reproduction are needed.

Acknowledgements

This work was supported by the National Fund for Scientific Research to Pr. N. Soltani (Laboratory of Applied Animal Biology) and the Ministry of High Education and Scientific Research of Algeria (CNEPRU Project to Dr. F. Tine-Djebbar).

References

1. Hardstone MC, Leichter C, Harrington LC, Kasai S, Tomita T, Scott JG. Cytochrome P450 monooxygenase-mediated permethrin resistance confers limited and larval specific cross-resistance in the Southern house mosquitoes, *Culex pipiens quinquefasciatus*. Pesticide Biochemistry and Physiology. 2007; 89:175-184.
2. Maha MS, Eman ET, El-Bahy MM. Molluscicidal and Mosquitocidal Activities of the Essential oils of *Thymus capitatus* Hoff. et Link. and *Marrubium vulgare* L. Revista do Instituto de Medicina Tropical de São Paulo. 2012; 54(5):281-6.
3. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian Journal of Medical Research. 2012; 135:581-598.
4. Chowdhury N, Chandra G. Mosquito larvicidal and antimicrobial activity of protein of *Solanum rillosum* leaves. BMC Complementary and Alternative Medicine, 2008; 8:6882-8862.
5. Kiran SR, Bhavani K, Sita DP, Rajeswara RBR, Janardhan KR. Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* DC against *Aedes aegypti* and *Anopheles stephensi.* Bioresource Technology. 2006; 97:2481-2484.
6. Arivoli S, Tennyson S, Martin JJ. Larvicidal efficacy of *Vernonia cinerea* (L.) (Asteraceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Biopesticides, 2011; 4:37-42.
7. Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus salgina* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du val. Journal of Stored Products Research. 2005; 41:91-102.
8. Sukumar K, Perich MJ, Boobar LW. Botanical derivatives in mosquito control: a review. Journal of the American Mosquito Control Association. 1991; 7:210-237.
9. Carvalho AFU, Melo VMM, Craveiro AA, Machado MILMB, Bantim Rabelo EF. Larvicidal activity of the essential oil from *Lippiasidoides* Cham. Against *Aedes aegepti* L. Memórias do Instituto Oswaldo Cruz. 2003; 98:569-71.
10. Cavalcanti ESBC, Morais SM, Lima MAA, Santana EWP. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti L.* Memórias do Instituto Oswaldo Cruz, 2004; 99 (5): 541–544.
11. Ansari MA, Mittal PK., Razdan RK, Sreehari U. Larvicidal and mosquito repellent activities of Pine *Pinus longifolia* (Family: Pinaceae) oil. Journal of Vector Borne Diseases, 2005; 42: 95- 99.
12. Traboulsi AF, Taoubi K, El-Haj S, Bessiere JM, Rammal S. Insecticidal properties of essential plant oils against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). Pest Management Science, 2002; 58(5): 491–5.
13. Vatandoost H, Sanei Dehkordi DA, Sadeghi SMT, Davari B, Karimian F, Abai MR, *et al.* Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima Mozaffarian* essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). Experimental Parasitology, 2012;

132(4):470-474.
14. Morales R. The history, botany and taxonomy of the genus *Thymus*. In E. Stahl-Biskup & F. Saez (Eds.). Thyme: The genus *Thymus*. *Medicinal and aromatic plants – Industrial profiles* London: Taylor & Francis, 2002; 13:978-0-203-21685-9.
15. Stahl-Biskup E. Essential oil chemistry of the genus *Thymu*s - a global view. In: Stahl-Biskup E, Saez F, editors. Thyme: The genus *Thymus* London: Francis & Taylor, 2002; 75–124.
16. Park YU, Koo HN, Kim GH. Chemical composition, larvicidal action, and adult repellency of *Thymus magnus* against *Aedes albopictus*. Journal of the American Mosquito Control Association. 2012; 28:192-8.
17. De la Torre Rodriguez YC, Martínez Estrada FR, Flores Suarez AE, Waksman de Torres N, Salazar-Aranda R. Larvicidal and cytotoxic activities of extracts from 11 native plants from North Eastern Mexico. Journal of Medical Entomology. 2013; 50:310-3.
18. Pitarokili D, Michaelakis A, Koliopoulos G, Giatropoulos A, Tzakou O. Chemical composition, larvicidal evaluation, and adult repellency of endemic Greek *Thymus* essential oils against the mosquito vector of West Nile virus. Parasitology Research, 2011; 109:425-430.
19. El Akhal F, El Ouali Lalami A, Ez Zoubi Y, Greche H, Guemmouh R. Chemical composition and larvicidal activity of essential oil of *Origanum majorana* (Lamiaceae) cultivated in Morocco against *Culex pipiens* (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2014; 4:746-50.
20. El Akhal F, Greche H, Ouazzani CF, Guemmouh R, El Ouali Lalami A. Chemical composition and larvicidal activity of *Culex pipiens* essential oil of *Thymus vulgaris* grown in Morocco. Journal of Materials and Environmental Science. 2015; 1:214-9.
21. El Akhal F, Guemmouh R, Maniar S, Taghzouti K, El Ouali Lalami A. Larvicidal activity of essential oils of *Thymus vulgaris* and *Origanum majorana* (Lamiaceae) against of the malaria vector *Anopheles labranchiae* (Diptera: Culicidae) original article. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(3):372-376.
22. Moharramipour S, Taghizadeh A, Meshkatalsadat MH, Talebi AA, Fathipour Y. Repellent and fumigant toxicity of essential oil from *Thymus persicus* against *Tribolium castaneum* and *Callosobruchus maculatus*. Communications in Agricultural and Applied Biological Sciences. 2008; 73:639-42.
23. Saroukolai AT, Moharramipour S, Meshkatalsadat MH. Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. Journal of Pesticide Science. 2010; 83:3-8.
24. Szczepanik M, Zawitowska B, Szumny A. Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). Allelopathy Journal. 2012; 30(1):129-142.
25. Popović A, Šućur J, Orčić D, Štrbac P. Effects of essential oil formulations on the adult insect *Tribolium castaneum* (herbst) (Col., Tenebrionidae). Journal of Central European Agriculture. 2013; 14(2):181-193.
26. Dahchar Z, Bendali-Saoudi F, Soltani N. Larvicidal activity of some plant extracts against two mosquito species *Culex pipiens* and *Culiseta longiareolata*. Journal of Entomology and Zoology Studies. 2016; 4(4):346-350.
27. Dris D, Tine-Djebbar F, Soltani N. *Lavandula dentata* essential oils : chemical composition and larvicidal activity against *Culiseta longiareolata* and *Culex pipiens* (Diptera: Culicidae). African Entomology, 2017; 25(2), in press.
28. Tine-Djebbar F, Bouabida H, Soltani N. Répartition spatio-temporelle des Culicidés dans la région de Tébessa. Editions Universitaires Européennes, 2016; *ISSN/ISBN*: 978-3-639-50856-7.
29. Jennings W, Shibamoto T. Qualitative analysis of flavour and fragrance volatile by glass capillary gas chromatography. New York Academic Press, 1980.
30. Adams RP. Identification of essential oil components by gas chromatograph/mass spectrometry. Carol Stream, USA: Allured Publishing Corporation, 1995.
31. Rehimi N, Soltani, N. Laboratory evaluation of Alsystyn, a chitin synthesis inhibitor, against *Culex pipiens pipiens* L. (Dip., Culicidae), effects on development and cuticle secretion. Journal of Applied Entomology. 1999; 123:437-441.
32. Boudjelida H, Bouaziz A, Soin T, Smagghe G, Soltani N. Effects of ecdysone agonist halofenozide against *Culex pipiens.* Pesticide Biochemistry and. Physiology. 2005; 83(2/3):115-123.
33. Anonym. Informal consultation on insect growth regulators, 1983; WHO/VBC/83.
34. WHO. Guidelines for laboratory and field-testing of mosquito larvicides, Ref. WHO/CDS/WHOPES/GCPP/ 13, 2005, 41.
35. Ozcan M, Chalchat JC. Aroma profile of *Thymus vulgaris L*. growing wild in Turkey. Bulgarian Journal of Plant Physiology. 2004; 30(3-4):68-73.
36. Imelouane B, Amhamdi H, Wathelet JP, Ankit M, Khedid K, El Bachiri A. Chemical composition of the essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. International Journal of Agriculture and Biology. 2009; 11:205-208.
37. Atti-Santos AC, Pansera MR, Paroul N, Atti-Serafini L, Moyna P. Seasonal Variation of Essential Oil Yield and Composition of *Thymus vulgaris* L. (Lamiaceae) from South Brazil. Journal of Essential Oil Research, 2004; 16:294-295.
38. Borugă O, Jianu C, Mişcă C, Goleţ I, Gruia AT, Horhat FG. *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. Journal of Medicine and Life. 2014; 3:56-60.
39. Hazzit M, Baaliouamer A, Verissimo AR, Faleiro ML, Miguel MG. Chemical composition and biological activities of Algerian *Thymus* oils. Food Chemistry. 2009; 116:714-721.
40. Svoboda KP, Hampson JB. Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, anti-inflammatory and other related pharmacological activities. 1999. *http://www.csl.gov.uv/ienica/seminars/*
41. Smallfield B. Introduction to growing herbs for essential oils, medicinal and culinary purposes. Crop & Food Research. 2001; 45:4.

42. Houmani Z, Azzoudj S, Naxakis G, Skoula M. The essential oil composition of Algerian Zaatar: *Origanum ssp*. and *Thymus ssp*. Journal of Herbs, Spices & Medicinal Plants, 2002; 9: 275–280.
43. Dob T, Dahmane D, Benabdelkader T, Chelghoum C. Composition and antimicrobial activity of the essential oil of *Thymus fontanesi*. Pharmaceutical Biology, 2006; 44(8):607-612.
44. Benayache F, Boureghda A, Ameddah S, Marchioni, E, Benayache S. Flavonoids from *Thymus numidicus* Poiret. Der Pharmacia Lettre. 2014; 6:50-54.
45. Msaada K, Salem N, Tammar S, Hammami M, Saharkhiz MJ, Debiche N *et al*. Essential Oil Composition of *Lavandula dentata*, *L. stoechas* and *L. multifida* Cultivated in Tunisia. Journal of Essential Oil Bearing Plants. 2012; 15(6):1030-1039.
46. Fachini-Queiroz FC, Kummer R, Estevao-Silva CF, Carvalho MDB, Cunha JM, Grespan R *et al.* Effects of Thymol and Carvacrol, Constituents of *Thymus vulgaris* L. Essential Oil, on the Inflammatory Response. Evidence-Based Complementary and Alternative Medicine, 2012, 657026.
47. Piccaglia R, Marotti M, Giovanelli E, Deans SG, Eaglesham E. Antibacterial and antioxidant properties of Mediterranean aromatic plants. Industrial Crops Production, 1993; 2:7-50.
48. Eman E, Aziz ST, Hendawi EDA, Omer EA. Effect of soil type and irrigation intervals on plant growth, essential oil yield and constituents of *Thymus vulgaris* plant. American-Eurasian Journal of Agricultural & Environmental Sciences. 2008; 4(4):443-450.
49. Tchoumbougnang F, Dongmo PMJ, Sameza ML, Mbanjo EGN, Tiako Fotso GB, Amvam Zollo PH *et al*. Activité larvicide sur *Anopheles gambiae* Giles et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun. Biotechnology, Agronomy, Society and Environment, 2009; 13(1):77-84.
50. Belaqziz R, Harrak R, Romane A, Oufdou K, El Alaoui El Fels MA. Antimicrobial and Insecticidal Activities of the Endemic *Thymus broussonetti* Boiss. and *Thymus maroccanus* Ball. Records of Natural Products, 2010; 4(4):230-237.
51. Maurya P, Sharma P, Mohan L, Batabyal L, Srivastava CN. Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *Anopheles stephensi* and *Culex quinquefasciatus*. Journal of Asia-Pacific Entomology. 2009; 12(2):113-115.
52. Sosan MB, Adewoyin FB, Adewunmi CO. Larvicidal properties of three indigenous plant oils on the mosquito *aedes aegypti*. Nigerian Journal of Natural Products and Medicine. 2001; 5:30-33.
53. Walied M Alarif, Zeinab S Abou-Elnaga, Seif-Eldin N Ayyad, Sultan S Al-lihaibi. Insecticidal Metabolites from the Green Alga *Caulerpa racemosa*. *CLEAN – Soil, Air, Water*, 2010; 38(5-6):548-557.
54. Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P. Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. Bioresource Technology. 2005; 96:1235-1240.
55. Tawatsin A, Asavadachanukorn P, Thavara U, Wongsinkongman P, Bansidhi J, Boonruad T *et al*. Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Ae. aegypti* (Diptera: Culicidae). Southeast Asian Journal of Tropical Medicine and Public Health. 2006; 37:915-931.
56. Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. Environment International, 2005; 31(8):1149-1166.