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Chemical Composition and Insecticidal Properties of *Thymus numidicus* (Poiret) Essential Oil from Algeria

Faiza Saidj ², Sid-Ahmed Rezzoug ³,
Fatiha Bentahar ², Chahrazed Boutekedjiret ^{1*}

¹Ecole Nationale Polytechnique, 10 avenue Hacen Badi, BP 182,
El Harrach, 16200 Alger, Algérie

²Laboratoire des Phénomènes de Transfert, Département de Génie des Procédés.
USTHB, BP 32 El Alia, Bab Ezzouar, 16111 Alger, Algérie

³Université de La Rochelle, Pôle Sciences, Laboratoire, ‘‘Maîtrise des
Technologies Agro-Industrielles’’, 17042 La Rochelle, France

Abstract: Essential oils of thyme (*Thymus numidicus* (Poiret)) from Algeria were isolated by steam distillation, analysed by gas chromatography coupled with the mass spectrometry (GC-MS) and investigated for their insecticidal activities against *Rhizopertha dominica*. Contact toxicity of global oil recovered after one hour of extraction and its fraction F1 recovered after 2.5 minutes of extraction were tested using the filter paper method. Analysis of the essential oil made it possible to identify 14 components, representing 75.4 % of the oil. The major components were thymol (51.0 %) followed by carvacrol (9.4 %), linalool (3.3 %), thymol-methyl-ether (3.2 %) and *iso*-caryophyllene (2.7 %). The evolution of the yield and the composition of essential oil as function of time extraction show that steam distillation is a very rapid process and the duration of extraction could be limited to 15 minutes, since more than 94 % of oil are extracted. The toxicity tests show that thyme oil and its fraction F1 have an insecticidal activity, nevertheless, the mortality obtained for global oil are weaker compared with those obtained for the F₁ fraction. This toxicity would be allotted to the presence in strong proportion of linalool and thymol.

Key Words: Thyme oil, *Thymus numidicus* (Poiret), chemical composition, insecticidal properties.

Introduction: Empirical at the beginning, the crop protection knew enormous progress with the discovery of the pesticides of synthesis. However, their massive and irrational use in the fight against the devastating insects of the cultures generated perverse effects such as: the development of phenomena of resistance at the treated devastating insects ^{1,2}, the pollution of the biosphere, the presence of residues of pesticides in the treated agricultural produce reaching inadmissible contents. In front of the extent of the problem, the methods of fights must be reconsidered. These last years, work was oriented towards the search for biopesticides biodegradable to replace the conventional pesticides. The use of the plants like source of pesticides was the several work object³⁻⁶. Many plants are recognized to have insecticidal properties. Tapondjou *et al.*^{7, 8} and Bouda *et al.*⁹, showed the toxic effect of essential oils of several plants (*Chenopodium ambrosioides* (L), *Eucalyptus saligna* (Smith), *Cupressus sempervirens* (L), *Ageratum conyzoides* (L), *Lantana camara* (L.) and *Chromolaena odorata* (L.) against the stored food product insects. Keita *et al.*¹⁰, showed that essential oils as well as the powders of *Ocimum basilicum* (L.) and *Ocimum gratissimum* (L.) are toxic against *Callosobruchus maculatus*. According to Hummelbrunner and Isman ¹¹, the components of many essential oils (thymol, carvacrol, *trans*-anethol, eugenol, (-)-terpineol) have a very high biocide effect on *Spodoptera litura* (Boisduval).

Thymus numidicus (Poiret) (Arabic Zaâteur) is an endemic species of Algerian and Tunisian North, Thymus kind of the family of *Labiata* 12. Many studies were interested in the chemical composition of essential oil of thyme¹³⁻¹⁷ like in its antibacterial^{18, 19}, antifungal¹⁹, antioxidant²⁰⁻²² and insecticidal properties. The present study was initiated to investigate the chemical composition and the contact toxicity of essential oil of *Thymus numidicus* (Poiret) from Algeria against *Rhizopertha dominica*, insect devastating of cereals.

Experiment

Plant material and extraction of essential oil: The vegetal matter used was collected from “Yakouren, Tizi Ouzou” area located 150 km East of Algiers in April 2004 and identified according to the Flora of Algeria 12. The thyme oil was recovered by steam distillation. The experimental tests have been carried out in laboratory scale, during one hour. 35 g of dry plant (3.25 % of moisture, steam flow rate of 6.3 g min⁻¹) was placed in a glass column, which the lower and higher parts were respectively connected to a steam generator and a condenser. The vapour produced by the steam generator crosses the plant, charged with essential oil then pass through the condenser to a receiving flask. The essential oil is collected, dried with anhydrous sodium sulphate and stored at 4°C until used. During distillation, plant was exposed to steam water at 100°C and 1 bar. Extractions were performed at least three times, and the mean values were reported.

The essential oil yield was estimated according to the dry vegetal matter. The moisture was determined by the toluene distillation method²³. In addition, in order to follow the evolution by time of the yield of essential oil and the relative content of some major components of oil, the essential oil were collected at different extraction periods and analysed.

Analysis: The essential oils were analysed by gas chromatography coupled to mass spectrometry (GC-MS) (Varian computerized system comprising a 3900 gas chromatograph coupled to a mass spectrometer model SATURN 2100 T) using two fused-silica-capillary columns with different stationary phases. The non-polar column was CP-Sil 8 (30m x 0.25mm x 0.25µm film thickness) and the polar one was a Stabilwax™ consisting of Carbowax™.

PEG (60m x 0.2mm x 0.25µm film thickness). GC-MS spectra were obtained using the following conditions: carrier gas He; flow rate 0.8 mL/min; split 1:30; injection volume 0.1 µL; injection temperature 220°C; oven temperature progress from 60°C during 10 min then rising to 250°C at 2°C/min and holding at 250°C for 20 min. The ionisation mode used was electronic impact at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas. Identification of the components was based on comparison of their relative retention indices (RRI) on apolar and polar columns, determined with reference to an homologous series of C₈–C₂₈ n-alkanes, and by a comparison of their mass spectral fragmentation patterns with those stored in the data bank (Wiley/NBS library) and the literature²⁴.

Insecticidal activity

Insects: *Rhizopertha dominica* were obtained from laboratory cultures maintained in the dark in incubator at 33°C and 70 % relative humidity.

Contact toxicity: A serial dilution of the essential oil of thyme recovered after 2.5 min noted F₁ fraction and global essential oil obtained after 1h was prepared in acetone. Contact toxicity was tested using filter paper impregnated method and the controls were determined using acetone. Filter papers (9.0 cm diameter) were impregnated with aliquots 5 ml of an appropriate concentration of essential oils or acetone (control). The filters were air dried for 5 min. 20 insects were confined to each filter paper within a glass ring (9.0 cm diameter, 1.5 cm). Fluon GPI was applied to the inner surface of each ring to prevent the insects from climbing onto the side of the ring. Five replicates were set up for each thyme oil concentrations and control. All insects were exposed to the filter paper treatment for 24 h, after which they were transferred to glass vial with culture media and kept in incubators at 33°C and 70 % relative humidity. Mortality was recorded daily until end-point mortality (when the number of

dead insects no longer increased with time) was reached after 6 days. Data were transformed using Shneider-Orelli formula given by :

$$M_c(\%) = \frac{M - M_t}{100 - M_t}$$

Where M_c is the corrected insect mortality, M the insect mortality in the treated population of insect and M_t the insect mortality in the control.

Results and discussion

Composition of the essential oil: The thyme essential oil has a movable liquid aspect, yellow colour and a pleasant aromatic and slightly spice odour. Analysis of the essential oil by GC-MS made it possible to identify 14 components whose rate is higher than 0.1 %, representing 75.4 % of the oil. These results are given in Table 1. As shown in this table, it can be seen that this oil was characterised by an important content in the oxygenated compound. Thymol and carvacrol were the main compounds with 51.0 % and 9.4 % respectively; linalool and thymol-methyl-ether which are the second more abundant compounds are present respectively at 3.3 % and 3.2 % and p-cymene present with 0.5 %, is the only identified monoterpene. Among the identified sesquiterpenes, the isocaryophyllene is most abundant with 2.7 %.

The predominance of phenolic compounds is in agreement with previous results reported from *T. numidicus* essential oil from Constantine (North-Eastern of Algeria)¹⁶ mainly represented with thymol (68.2 %), carvacrol (16.9 %) and linalool (11.5 %); and *T. numidicus* essential oil from Berrahal (area located 500 km East of Algiers)¹⁵, which was characterized by thymol (60.8 %) as the major component, followed by linalool (8.0 %) and carvacrol (5.1 %). In addition, we notice that the amount of linalool in our sample is weaker than that of oils from Constantine and Berrahal, however it is comparable with that of oil from Souk-Ahras (area located 650 km East of Algiers)¹⁵ characterized by thymol (25.4 %) and carvacrol (11.3 %) as the major component, followed by linalool (2.7 %).

The difference in the composition of essential thyme oil is very frequent. Faleiro *et al.*²³ report a similar behaviour for three Portuguese thymes (*T. comphoratus*, *T. lotocephatus* and *T. mastichina*) and by Rasooli and Abyanet¹³ for two species of thyme collected in Iran (*T. eriocalyx* and *T. x-porlock*). Indeed, the climatic and environmental factors, the intensity of the metabolism of the plants, the species, the age and the part of plant, the period and the area of the gathering as well as the activity of the glandular hairs affect the synthesis and the secretion of oils.

Yield and essential oil composition as function of time: The yield of essential oil obtained by the steam distillation is 2.06 %. This value is comparable with that given in the literature, especially with that given by Kabouche *et al.*¹⁶ for *T. numidicus* from Djebel El- Ouahch mountain, Constantine. Evolution of essential oil yield as function of time, figure 1, shows that the extraction time is relatively short since at the end of one hour, we do not observe any more essential oil in the distillate. The yield increases quickly during the first 15 minutes were more than 94 % of essential oil are recovered. Thereafter, its evolution becomes slower; after 60 minutes, it is negligible. The analysis by gas chromatography of the oil recovered with regular time intervals enabled us to follow the evolution by time of the relative content of some major components of oil belonging to different chemical families : linalool, thymol-methyl-ether, thymol, carvacrol and *iso*-caryophyllene. As it is shown in table 2, we notice that the considered components are recovered in the ascending order of their boiling points. Indeed, the linalool and the thymol-methyl-ether were mostly recovered after 2.5 minutes of the extraction process with 8.2 % and 3.0 % respectively. The thymol reach their maximum contents (79.3 %) after five minutes of extraction and finally the carvacrol and *iso*-caryophyllene follows them after ten minutes of steam distillation with a maximum content of (12.2 %) and (4.2 %) respectively. The fact that the considered components are recovered in the ascending order of their boiling points lets

suppose that the steam distillation depends on phenomena of transfer of the oil located at the surface of the plant and of its evaporation. Besides, the present study, showed an interest: this way of extracting the oil (fractionnement) allow obtaining the essential oil of different qualities enriched with particular components. It also may be used for analytical purposes or to avoid additional oil processing such as distillation and rectification.

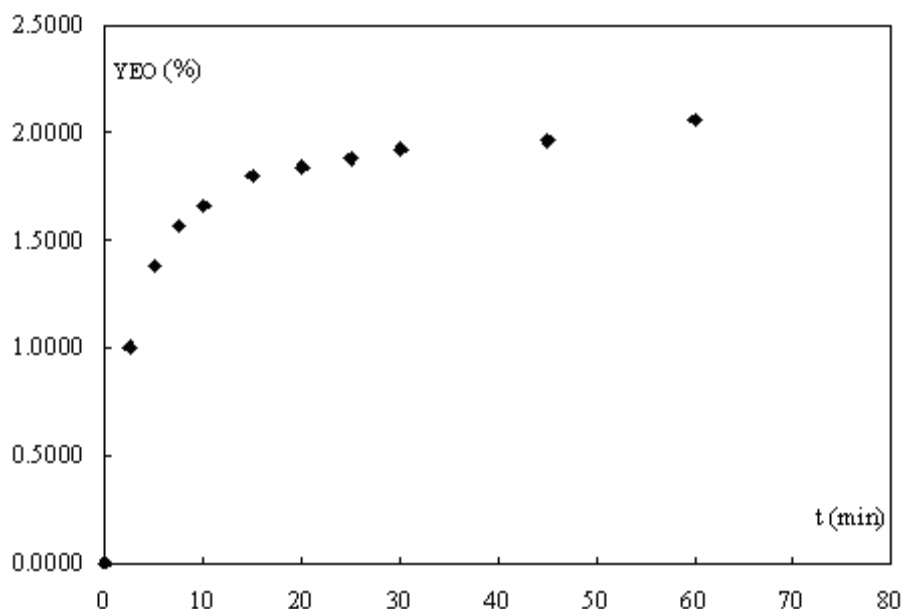


Figure 1. Evolution of the essential oil yield as function of time

Insecticidal activity: The results of toxicity tests are gathered in table 3, its shows increase in the insect mortality with the concentrations of used solutions of essential oils. The mortality obtained for global oil are weaker compared with those obtained for the F_1 fraction. For a concentration of 3.125 (%V) the oil recovered after 2.5 min involves a percentage of mortality of 86.73 % against 79.59 % for oil extracted after 1 hour; 94.86 % of mortality are obtained for a concentration of 12.5 (%V) in F_1 fraction, whereas global essential oil causes only 87.69 %. These results show that the F_1 fraction is more efficient against *Rhizopertha dominica* than the global oil. These behaviours could be explained by the content of major compounds of these oils (Table 4). Indeed, the percentage of thymol and linalool are more significant in F_1 fraction, whereas the amount of thymol-methyl-ether and carvacrol is practically the same. These observations would suggest that toxicity by contact effect of F_1 fraction would be allotted to the presence in strong proportion of linalool and thymol. Nevertheless, the additional tests using of the solutions of pure linalool and thymol and the mixtures with various concentrations are to be planned to confirm or cancel these assumptions.

Conclusion: This study enabled us to determine the chemical composition of the essential oil of *Thymus numidicus* (Poiret) from Algeria, extracted by steam distillation and to evaluate its insecticidal activity and that of its fraction recovered after 2.5 minutes of extraction against *Rhizopertha dominica*, a devastating insect of cereals. Analysis of the essential oil by GC-MS made it possible to identify 14 components whose rate is higher than 0.1 %, representing 75.4 % of the oil. The major components were thymol (51.0 %) followed by carvacrol (9.4 %), linalool (3.3 %), thymol-methyl-ether (3.2 %) and *iso*-caryophyllene (2.7 %). The evolution of the yield and the composition of essential oil as function of time extraction show that steam distillation is a very rapid process which seems to be regulated by essential oil evaporation phenomena from the superficial sites of the plant. In addition, the results obtained show that the duration of extraction could be limited to 15 minutes, since

more than 94 % of oil are extracted. The toxicity tests show that thyme oil and its fraction F₁ have an insecticidal activity. Indeed, the mortality of the insect increases with the concentration of oils used nevertheless, the mortality obtained for global oil are weaker compared with those obtained for the F₁ fraction. This toxicity would be allotted to the presence in strong proportion of linalool and thymol. These results suggest that thyme oil and its fraction may be useful as a grain protecting with contact effect against *Rhizopertha dominica* and could replace synthetics insecticides.

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Table 1. Chemical composition of essential oil of *Thymus numidicus* (Poiret) Compound RI (%)

Compound	RI	(%)
Octen-3-ol	978	0.5
p-Cymene	1026	0.5
Linalool	1098	3.3
α -Terpineol	1189	0.4
Thymol-methyl-ether	1235	3.2
Thymo-quinone	1249	0.4
Thymol	1290	51.0
Carvacrol	1298	9.4
β -Caryophyllene	1404	0.9
<i>iso</i> -Caryophyllene	1438	2.7
γ -Cadinene	1513	0.3
δ -Cadinene	1524	0.5
α -Cadinene	1538	0.5
Caryophyllene-oxyde	1581	1.8
Total		75.4

Table 2. Evolution of relative content of major component of essential oil as function of time extraction

Time (min)	Relative content (%)				
	Oxygenated Linalool	Monoterpene Thymol-methyl-ether	Phenolic Thymol	Monoterpene Carvacrol	Sesquiterpene Isocaryophyllene
2.5	8.2	3.0	74.3	8.6	0.0
	Total : 11.2		Total : 82.9		Total : 0.0
5	3.5	0.8	79.3	11.9	1.8
	Total : 4.3		Total : 91.2		Total : 1.8
7.5	1.8	0.7	76.0	11.5	2.4
	Total : 2.5		Total : 87.5		Total : 2.4
10	*tr < 0.05 %	tr	71.0	12.2	4.2
	Total : tr		Total : 83.2		Total : 4.2
20	tr	tr	31.1	tr	0.0
	Total : tr		Total : 31.1		Total : 0.0
45	0.0	tr	32.9	tr	0.0
	Total : tr		Total : 32.9		Total : 0.0
60	0.0	0.0	tr	tr	0.0
	Total : 0.0		Total : tr		Total : 0.0

*tr : traces < 0.05%

Table 3. Contact toxicity of global thyme essential oil and its fraction Corrected Mortality : M_c (%)

C_{EO} (%V)	global EO (0 – 60 min)	Fraction F_1 (0 – 2.5 min)
Control	0	0
3.125	79.59 ± 3.28	86.73 ± 2.80
6.250	80.61 ± 2.39	93.33 ± 1.19
12.500	84.69 ± 2.45	94.86 ± 1.57

Table 4. Content of major components of thyme oil and its fraction

Sample	Linalool	Thymol-methyl - ether	Thymol	Carvacrol
Global EO (0 – 60 min)	3.3	3.2	51.0	9.4
Fraction F_1 (0 – 2.5 min)	8.2	3.0	74.3	8.6