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Extraction of *Thymus pallecens* de Noé essential oil by microwave steam distillation and steam distillation processes: Kinetics, optimization, chemical composition and insecticidal activity

S. Bertouche[1], N. Sabba[1], N. Sahraoui[1]*, Y.Nait Bachir[2]

[1] Laboratory of valorization and recycling of matters and sustainable development, Faculty of Mechanical Engineering and Process Engineering USTHB, BP 32, Algiers, Algeria
[2] Chemical Engineering Laboratory, Process Engineering Department, Faculty of Technology, University of Saad Dahlab-Blida1

*Corresponding author: sahraouinaima@yahoo.fr;

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ABSTRACT/RESUME

Abstract: *The essential oil of Thymus pallescens de Noé has been isolated using an extraction process developed in our laboratory: steam distillation assisted by microwaves also called microwave steam distillation (MSD). This process is a combination of traditional techniques, namely, a steam distillation (SD) and an innovative technology, a microwave heating. Kinetic study of extraction, analysis of essential oils by gas chromatography and mass spectrometry, optimization of operating conditions using response surface methodology (RSM) were studied. The selected operating parameters are the steam flow rate and the microwave heating power. Yield obtained by microwave steam distillation is comparable to that obtained by the conventional steam distillation, while the extraction time is greatly reduced: 6 min for MSD extraction against 15 min for the SD. The best performance was obtained with a power of 600Wand steam flow rate of 10 g•min-1. The essential oils of T. pallescens isolated either by MSD or by SD are rather similar in their composition. The same number of components is found in the essential oils with equivalent relative amounts for both extraction methods. The contact and fumigation toxicities of these oils were tested against the insect Rhyzopertha dominica using the filter paper method. The toxicity tests showed that insect mortality increased with the concentration used (0.39%, 0.78%, 1.56% and 3.12%V). The results suggest that the oil T. pallescens has important insecticidal properties and may be useful as a natural cereal protection agent against Rhyzopertha dominica to replace synthetic insecticides*

I. Introduction

For thousands years, mankind has used various plants found in its environment to treat and cure all kinds of diseases. These plants show a great diversity of chemical structure and possess a very wide range of biological activities. The evaluation of these activities remains a very interesting task that could be the focus of many studies [1-3]. The popularity of aromatic plants in general and their essential oils in particular, remains linked to their medicinal properties including anti-inflammatory, antiseptic, antiviral, antifungal, bactericidal, antitoxic, insecticidal, antioxidant, stimulant, and calming ones. Moreover, these plants have always occupied an important place in the composition of perfumes and in culinary preparations [4].

Conventional techniques for extraction or isolation of chemical compounds from plant matrices often present constraints such as low yields, very long

extraction times and use of large quantities of solvents. Essential oil extraction processes using microwaves are now well known to have significant effects on extraction kinetics and on the reduction of extraction time [5-10]. Indeed, the result of using this process is an improvement in yield, particularly regarding solid-liquid extraction and the quality of the recovered products.

The present study aims firstly to investigate the impact of operating conditions and the efficiency of the (MSD) technique for the extraction of *T. pallescens* essential oil using a response surface methodology (RSM). It was realized using a centered composite design (CCD). This method is used in several studies to evaluate the performance of processes [5, 7, 11, 12] by reducing the number of trials needed to evaluate the effect of several parameters and their interactions.

The second objective is to evaluate and compare the insecticidal activity of essential oils obtained by (MSD) and (SD). The essential oil of *T. pallescens*, has been tested against the insect *Rhyzopertha dominica* which devastates stored cereals.

II. Materials and methods

II.1. Extraction Procedure

II.1.1. Plant Material

The plant material used for this study was collected in April 2018 from Blida area located about 45 km south-west of Algiers (Algeria). The taxonomic identity of the plant as T. pallescens was accomplished and identified according to the flora of Algeria [13,14], and confirmed by comparison with specimen of known identity already deposited in the herbarium of the Agronomic Higher National School of Algiers (ENSA).

The leaves were air-dried, and the moisture content, determined by Dean-Stark method [15], was 8, 98%.

II.1.2. Apparatus and Procedure

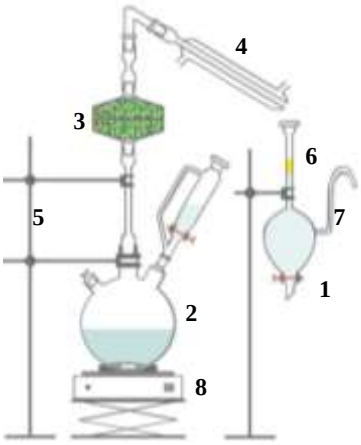
The oil was recovered from *T. Pallescens* leaves by MSD and conventional steam distillation (SD). Essential oil yield (YEO) was estimated according to dry vegetable matter, and expressed in g of EO per 100 g of dry matter.

II.1.3. Microwave Steam Distillation Apparatus and Procedure

MSD apparatus (Fifure1a) is a multimode microwave reactor operating at 2.45 GHz with a maximum delivered power of 1,000 W variable in 10 W increments. During experiment time, temperature and power were recorded and controlled. An electrical steam generator and a condenser placed outside the microwave oven are connected to a cartridge containing the plant via Pyrex connecting tubes. The condenser is connected to a receiving Florentine flask which is preferably a separating funnel to enable the continuous collection of condensate essential oil and water. The cartridge containing 12 g of the plant is subjected to microwave heating; steam passes through the sample, evaporates, and carries the essential oil directed toward the condenser and the Florentine flask. The extraction was continued until no more essential oil was obtained [1, 2, 5]. The essential oil is collected, dried with anhydrous sodium sulfate, and stored at 4°C until used. Extractions were performed at least three times, and the mean values were reported.

II.1.4. Steam Distillation Apparatus and Procedure

For rigorous comparison, the same glassware and same operating conditions have been used for conventional steam distillation (the same process but without the use of microwaves). In this system (Figure 1b), the vapor produced by the steam generator crosses the plant, charged with the essential oil, and then passes through the condenser to a receiving Florentine flask. The extraction was continued until no more essential oil was obtained. The essential oil is collected, dried with anhydrous sodium sulfate, and stored at 4°C until used. Extractions were performed at least three times, and the mean values were reported.



1- Florentine vase
2- Balloon water
3- Glass column + Plant material
4- Condenser
5- Stand
6- EOs
7- Water
8- Heating source
9- Microwave oven

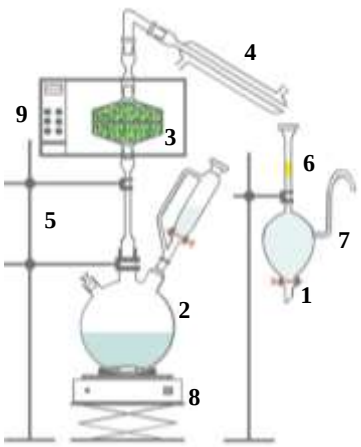


Figure 1a. *Steam distillation apparatus*

Figure 1b. *Microwave steam distillation apparatus*

II.2. Analysis

Essential oils obtained by both MSD and SD are analyzed by gas chromatography coupled to mass spectrometry (GC/MS) using a Hewlett-Packard computerized system comprising a 6890 gas chromatograph coupled to a 5973A mass spectrometer. A fused silica-capillary columns HP5MS (30 m × 0.25 mm, 0.25 µm film thickness) was used. GC/MS spectra are obtained using the following conditions: carrier gas He; flow rate 0.3 mL min-1; splitless mode; injection volume 1 µL; injection temperature 250 °C; oven temperature program is 60°C for 8 min, increased at 2°C min-1 to 250 °C and held at 250 °C for 15 min. The ionization mode used is electron impact at 70 eV. The relative percentage of the components is calculated from GC with flame ionization detection (GC–FID). Most constituents are tentatively identified by comparison of their GC Kovats retention indices (RI), determined with reference to a homologous series of C9-C17 n-alkanes. Some structures were confirmed by authentic standards available in the laboratory and analyzed under the same conditions described above. Identification is confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS database and with mass spectra literature data [16, 17].

II.3. Physical constants

T. pallescens essential oils have been analyzed according to the standard method AFNOR [18]. The usual physical, chemical and organoleptic constants defining the essential oil have been determined at 20°C: specific gravity, refractive index, acid number, ethanol miscibility, aspect, colour and odour.

II.4. Optimization of extraction

To investigate the performance of MSD process, an optimization of the operating conditions was achieved. The operational parameters chosen were microwave power (P), steam water flow rate (Q) and processing time (t). Optimization was carried out by a parametric study and an experimental design.
For a parametric study, several flow rates (4; 6; 8; 10 and 12 g.min-1) and microwave irradiation powers (100; 200; 300; 400; 500; 600; 700 and 800 W), were examined.

II.4.1. Experimental design

A Box–Wilson procedure, also known as Central Composite Design (CCD), was used to achieve maximal information of the process with a minimal number of possible experiments. The multivariate study allows the identification of interaction between variables and provides a complete exploration of the studied experimental domain. The type of CCD used in this study was Central composite face-centered (CCF) experimental design to determine the optimal conditions of MSD process. The application of a CCF design is a convenient way to optimize a process with five levels (-α,-1, 0, +1, +α) for each factor. In this design, the star points are at the centre of each face of the factorial space, thus ± α = ±1 [19, 20]. A total of 18 different combinations, including 23 full factorial designs (±1) with four axial points (± α) and four replicates of center point (coded 0), were investigated to fit the full quadratic equation model given by equation (1).

$$Y_{EO} = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad \textbf{(1)}$$

Where Y_{EO} represented the response variable (essential oil yield in this study), β_0, β_i, β_{ii}, and β_{ij} were the regression coefficients of variables for intercept, linearity, square, and interaction terms, respectively. X_i and X_j were the independent coded variables influencing the response variable Y_{EO}, and k represents the quantity of variables. The range of the studied parameters was chosen based on a parametric study. Data obtained from the CCD were analyzed statistically with the software Statgraphics plus (Version 5.1, Statistical Graphics Corporation, Rockville, USA, 2000)

II.5. Insecticidal activity

A serial dilution of the essential oil of recovered by both MSD and SD was prepared in acetone. The filter paper impregnated method was used to examine contact and fumigant toxicity, and the controls were determined using acetone.

II.5.1. Insects

Rhyzopertha dominica were obtained from laboratory cultures maintained in the dark in an incubator at 33 °C and 70% relative humidity.

II.5.2. Contact toxicity

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. 25 insects were confined to each filter paper within a glass ring (9.0 cm diameter). Fluon GPI was applied to the inner surface of each ring to prevent the insects from climbing onto the

side of the ring. 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.

II.5.3. Fumigant toxicity

Filter papers (3.0 cm diameter) were impregnated with aliquots 2.5 mL of an appropriate concentration of essential oils or acetone (control). The filters were air dried for 5 min; then attached to the undersurface of the screw cap of a glass vial. The cap was screwed tightly onto the vial containing 25 insects. After 24 h, the insects were transferred to clean vials with culture media and kept in incubators at 33°C and 70 % relative humidity. Mortality was recorded daily until end-point mortality was reached after 6 days.

Five replicates were set up for all tests in both contact and fumigant toxicity, and mortality were calculated using Shneider-Orelli relation given by:

$$M_C(\%) = \left[M - M_t / 100 - M_t\right] \quad \textbf{(2)}$$

Where, Mc is the corrected insect mortality, M the insect mortality in the treated population of insect and Mt the insect mortality in the control.

Bioassays were designed to assess respectively median lethal concentration LD_{50} and LD_{90} values, doses that kill 50 % and 90 % of the exposed insects. Probity analysis was conducted to estimate these lethal doses [21].

III. Results and discussion

III.1. Extraction yield and time

Variations yields of *T. pallescens* oil as a function of time (Fifure 2) show differences in the kinetics of the two processes and confirm the speed of extraction by MSD.

Indeed, the yields obtained by the studied processes are comparable: 3.60 ± 0.25 % and 3.63 ± 0.25 % for MSD and SD, respectively, the only difference observed is the duration of extraction. An extraction time of 6 min with MSD provides a yield comparable to that obtained after 15 min by SD.

The extraction kinetics of *T. pallescens* essential oils extracted by MSD show a single step. The curve can be divided into two parts:

- 1st increasing part: in which the yield increases rapidly and all the essential oil is recovered, suggesting that the essential oil glands distend and burst fast under the effect of microwaves to let the essential oil escape.
- 2nd stable part: where the yield is constant, which means that the extraction process is finished.

For the process SD the corresponding curve for each plant can be divided into three parts:

- 1st increasing part (fast): in which we observe a rapid increase in yield due to the water vapour generated which causes the cells burst and release the essential oil.
- 2nd growing part (slow): where we observe a slowing of the extraction which means the exhaustion of the essential oil contained in the plant cells.
- 3rd stable part: during which the yield is constant which corresponds to the end of the process.

The study of the extraction kinetics highlighted the efficiency of MSD in terms of time for comparable yields.

These results are in accordance with those obtained by Sahraoui et al. for the essential oils extraction of lavender flowers, orange peels and oregano leaves [1,2,5] and confirm that one of the advantages of the (MSD) method is its rapidity.

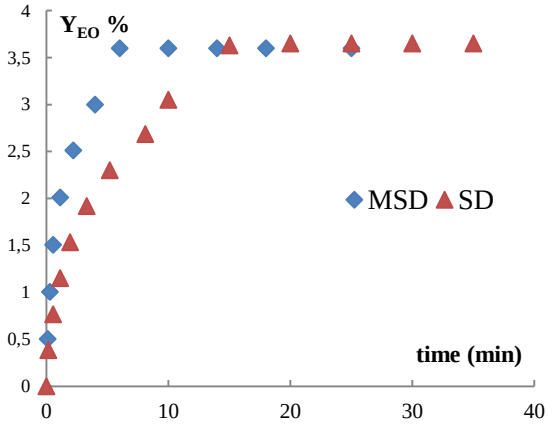


Figure 2. *Profile of essential oil yield obtained by MSD and SD as a function of extraction time.*

III.2. Characterization of essential oils

In order to determine the usual organoleptic, physical and chemical constants characterizing the essential oil extracted by MSD and SD, the *T. pallescens* plant essential oils were analyzed according to the AFNOR standard method (2000) [18]. The results are given in Table 1.

III.2.1. Physico-chemical and organoleptic properties of the essential oil of thyme

The comparison of the physico-chemical and organoleptic properties of the essential oils of the thyme extracted by the two methods MSD and SD in (Table 1) shows that organoleptic properties of the two essential oils are the same.

On the other hand, regarding the physico-chemical properties, a very slight difference can be noticed. Thus, MSD does not alter or influence the quality of the extracted oils.

Table 1. *Physico-chemical and organoleptic properties of the essential oil of thyme obtained by MSD and SD*

Process		MSD	SD
Physical constants	**Density at 20°C**	0.935	0.925
	Refractive index at 20°C	1.491	1.51
Chemical constants	**Acid number**	1.59	1.59
	95% ethanol miscibility	1V/1V	1V/1V
Organoleptic characterization	**Aspect**	Mobile liquid	Mobile liquid
	Colour	light brown	light brown
	Odour	Characteristic, aromatic, slightly spicy.	Characteristic, aromatic, slightly spicy

III.2.2. Chemical composition

Thymus essential oils obtained by MSD and SD were analyzed by GC and GC-MS (Table 2) and compared in terms of chemical composition and relative amount of each component. The results show that the studied Thymus species is mainly composed by carvacrol (92.63-83.8 %) respectively for microwave assisted steam distillation and steam distillation.

On the other hand, the content of other compounds varies between (0.28 - 0.01 %) for both processes, except for: para-cymene at (4.45-8.15 %), linalool at (0.9 -1.44 %), and gamma- terpinene at (1.01- 4.96 %) respectively for MSD and SD.

The chemical composition by family of the essential oil of thyme reveals that:

- The monoterpenes are low in both processes;
- Both oils are characterized by very high levels of oxygenated monoterpenes;

The essential oils extracted by MSD and SD have very low levels of sesquiterpenes and an absence of oxygenated sesquiterpenes;

In addition, other oxygenated compounds of low content are present in both oils (table 2).

Table 2. *Chemical composition of T. pallescens EOs obtained by MSD and SD*

N°	Compounds[a]	LRI[b]	ERI[c]		Relative content (%)		Identification methods[d]
			MSD	SD	MSD	SD	
	Monoterpenes				**5.62**	**13.5**	
1	α-Terpinene	1018	1019	1020	0.11	0.28	MS, Co-GC
2	p-Cymene	1026	1028	1026	4.45	8.15	
3	trans-β-Ocimene	1050	1052	1051	0.05	0.11	
4	γ-Terpinene	1062	1065	1057	1.01	4.96	RI, MS, Co-GC
	Oxygenated monoterpenes				**93.74**	**85.71**	
5	Linalool	1098	1123	1104	0.9	1.44	RI, MS, Co-GC
6	Terpin-4-ol	1177	1179	1179	tr	0.05	RI, MS, Co-GC
7	Carvacrol methyl ether	1244	1282	1245	0.12	0.19	RI, MS
8	Thymol	1290	1302	1296	0.21	0.23	RI, MS, Co-GC
9	Carvacrol	1298	1318	1343	92.63	83.8	RI, MS, Co-GC
	Sesquiterpenes				**0.11**	**0.13**	
10	Aromadendrene	1439	1439	1445	0.11	0.06	RI, MS
11	α-Humelene	1454	1454	1448	tr	0.03	RI, MS
12	γ-Cadinene	1513	1513	1520	tr	0.01	RI, MS
13	δ-Cadinene	1524	1542	1529	tr	0.03	RI, MS
	Other oxygenated compounds				**0.12**	**0.19**	
	Total oxygenated monoterpenes compounds				**93.86**	**85.71**	
	Total Monoterpenes and sequiterpenes hydrocarbons				**05.73**	**13.63**	
	Total components				**99.59**	**99.34**	

Legend:
MSD: Microwave steam distillation.
SD: Steam distillation.
[a] Components quantified on non-polar column HP 5MS capillary column and listed in order of elution from the same column.
[b] Literature retention indices relative to non-polar column HP 5MS [16,17].
[c] Experimental retention indices relative to n-alkanes C9–C17 on non-polar column HP 5MS.
[d] Identification: co-GC, comparison with authentic compounds; MS, comparison of mass spectra with MS libraries; RI, comparison of retention index with bibliography.

III.3. Optimization results

III.3.1. Parametric study

In order to carry out this study, we evaluated the influence of two parameters: The steam flow rate and the microwave heating power. The plant material was set at 12g for all tests. Trials were carried out for different flow rates (4; 6; 8; 10 and 12 g.min-1) to evaluate its influence on the essential oil yield. The results are shown in Table 3 and represented in (Figure 3). We note that the yield of essential oil of *T. pallescens* increases from 2.17 to 3.81 when the flow rate increases from 4 to 10 g.min-1 and decreases to 2.34 for a flow rate of 12 g.min-1. A high flow can involve the creation of a by-pass, having for consequence a reduced contact between the vapor and the plant and thus decreasing the yield. A low flow would be insufficient to recover all essential oil. The optimum was obtained with steam flow of 10 g min-1.

Thyme essential oil yield evolution increases up to an optimum value with the power augmentation. The results are shown in Table 3 and represented in Figure 4. Thus, when the power increases from 100 to 900 W, the yield of essential oil increases from 2.30 ± 0.05 % to 2.60 ± 0.06 %, respectively, and then decreases for the rest of the power values. An appropriate microwave irradiation power is important to ensure that the essential oil is extracted quickly; however, the power should not be too high so that would induce the destruction of the vegetable matter. The best performance is obtained for 600 W.

Table 3. *Results of a parametric study*

Operating conditions	Q (g·min^{-1})	Yield (%)	P(W)	Yield (%) Q = 10 g.min^{-1}
	4	2.17	100	2.30
Plant material mass	6	2.80	200	2.61
m = 12g	8	3.07	300	2.81
	10	**3.81**	400	2.90
	12	2.34	500	3.02
Moisture content			600	**3.52**
H = 8.982 %			700	2.82
			800	2.60

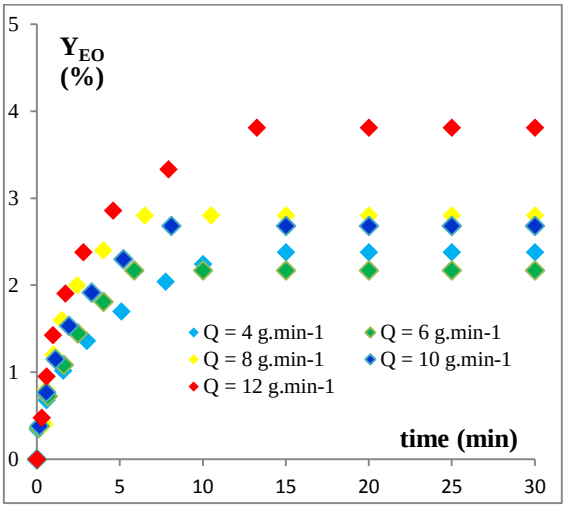


Figure 3. *Yield of essential oil as a function of vapor flow.*

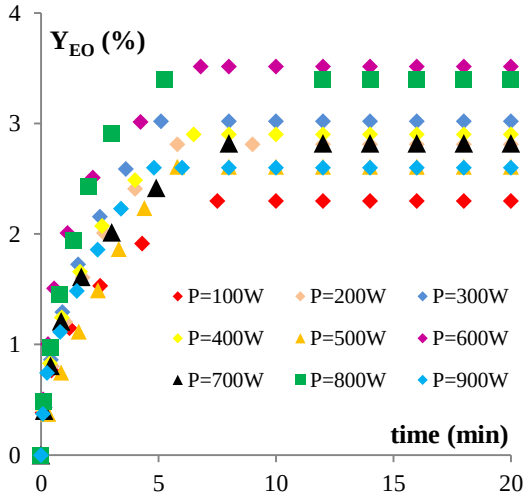


Figure 4. *Yield of essential oil as a function of power.*

In conclusion, parametric study showed that the best performance is obtained with a power of 600 W and a steam flow rate of 10 g min^{-1}.

III.3.2. Central composite design results

Table 4 shows the experimental design matrix and the responses obtained in the multivariate study for the 18 trials programmed regarding extraction by microwave steam distillation. From those results, we note that trial number 12 offers the optimal yield of essential oil (3.52%) which corresponds to the coordinate values:

Reduced: (0, 0, 0).

Real: Q = 10 g.min-1; P = 600 W; t = 6 min.

These results are in accordance with those obtained by the parametric study.

In addition, the values obtained for the tests corresponding to the repetitions in the center show a good reproducibility of the results.

Table 4. *Results of central composite design for essential oil extracted by MSD*

	Real variables			Coded variables			Y_{EO} (%)
Runs	Flow rate (g min^{-1})	Power (W)	Time (min)	**A**	**B**	**C**	
1	8	400	4	-1	-1	-1	2.20
2	12	400	4	+1	-1	-1	2.79
3	8	800	4	-1	+1	-1	2.45
4	12	800	4	+1	+1	-1	2.77
5	8	400	8	-1	-1	+1	2.54
6	12	400	8	+1	-1	+1	2.04
7	8	800	8	-1	+1	+1	3.23
8	12	800	8	+1	+1	+1	2.35
9	10	600	6	0	0	0	3.14
10	10	600	6	0	0	0	3.16
11	10	600	6	0	0	0	3.40
12	**10**	**600**	**6**	0	0	0	**3.52**
13	12	600	6	+1	0	0	2.28
14	8	600	6	-1	0	0	2.63
15	10	800	6	0	+1	0	3.40
16	10	400	6	0	-1	0	2.89
17	10	600	8	0	0	+1	3.52
18	10	600	4	0	0	-1	2.95

Note: The values in bold and underlined mean correspond to the optimal conditions obtained by RSM study.

Analysis of variance (ANOVA) was performed on the design to assess its significance which depends on the number of degrees of freedom (DF) in the model, and is shown in the P-value column (95% confidence level) (table 5). Therefore, the effects lower than 0.05 in this column are significant. This is emphasized by the standardized Pareto chart in Figure 5, which reveals three significant coefficients affecting the extraction (within the chosen limits), namely, quadratic steam flow - steam flow effect (AA), time-flow interaction effects (AC) and irradiation power.

Table 5. *Analysis of variance (ANOVA)*

Parameter	**P-Val**
A : flow	0.2259
B : Power	**0.0245**
C : time	0.4389
AA	**0.0003**
AB	0.2608
AC	**0.0035**
BB	0.7643
BC	0.2077
CC	0.6768

Statistical analysis of CCD results made it possible to determine an empirical relationship linking studied response (Y_{EO}) and key variables involved in the model, which is described by the following polynomial equation of fitted model:

$$Y_{EO}\ \mathbf{(\%)} = 3.24219 - 0.1646\mathbf{Q} + 0.3468\mathbf{P} + 0.1022\mathbf{t} - 1.45376\mathbf{Q^2} - 0.16975\mathbf{QP} - 0.57225\mathbf{Qt} - 0.0747619\mathbf{P^2} + 0.19225\mathbf{Pt} + 0.104238\mathbf{t^2} \quad \mathbf{(3)}$$

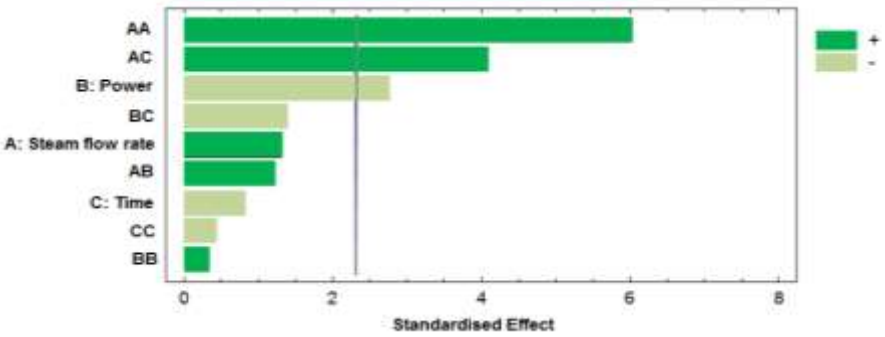


Figure 5. *Pareto chart*

The simple effects of flow and time, and also of power-time, power-flow, time-time and power-power interaction being insignificant, the above equations can be reduced to:

$$Y_{EO}\ \mathbf{(\%)} = 3.24219 + 0.3468\mathbf{P} - 1.45376\mathbf{Q^2} - 0.57225\mathbf{Qt} \quad \mathbf{(4)}$$

Where (Y_{EO}) denotes essential oil yield (%), Q steam flow (g min^{-1}), P microwave power (W) and t extraction time (min). The coefficients of this model express the effect of these variables on the essential oil yield. A positive value supports the operation, whereas the negative values disadvantage it.

The coefficient of determination of the model R^2 indicating the goodness of fit of the estimates for the regression equation, we considered for our study.

In our study, the R^2 coefficient is equal to 91.716% ($0 < R^2 < 1$) which means a good fit of the model and a good description of the system studied in the experimental domain.
This model also allowed us to determine the optimal points for each variable and compare it with the experimental values (table 6).

Table 6. *Experimental and statistical estimated optimal operating conditions*

Optimal operating conditions	
Experimental	Estimated
Q = 10 $g.min^{-1}$	Q = 9.35 $g.min^{-1}$
P = 600 W	P = 785.802 W
t = 6 min	t = 8.0 min
Y_{EO} = 3.52 %	Y_{EO} = 3.63 %

From Table 6 we can see that the values of the experimental and model-estimated optimal conditions are comparable for the flow rate, while for the power, the theoretical values are higher and approach the values of the upper level (800 W). Regarding the time, its theoretical value is identical to the corresponding value of the upper level which is 8 min, difference that may be due to experimental errors.

Response surfaces are three-dimensional graphical representations of the understudy system response as a function of two parameters, the third being fixed at its central level. They are used to search for experimental regions for which the response is optimal.

Figure 6 representing the yield of essential oil variations as a function of the flow-time pair shows that the optimal yield is in the region of the space corresponding to the values of the experimental coordinate range (- 0.2; +1) in reduced variables and to (9.2 $g.min^{-1}$; 8 min) in real variables.

Figure 7 representing the yield of essential oil variations as a function of the flow-power pair shows that the optimal yield is in the area of the space corresponding to the values of the experimental coordinate range (- 0.3; + 0.8) in reduced variables and to (9,4 $g.min^{-1}$; 760 W) in real variables.

Figure 8 representing the yield of essential oil variations as a function of the Power-time pair shows that the optimal yield lies in the area of the space corresponding to the values of the experimental coordinate range (+1; +1) in reduced variables and to (8 min; 800 W) in real variables.

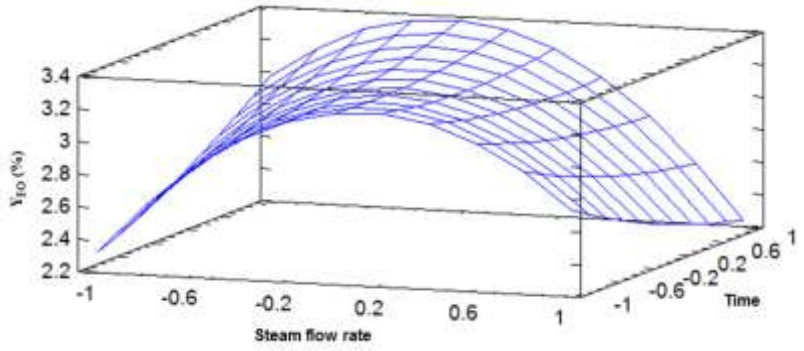


Figure 6. *Response Surface Plots for T. pallescens essential oil depending on the flow-time for P = 600 W.*

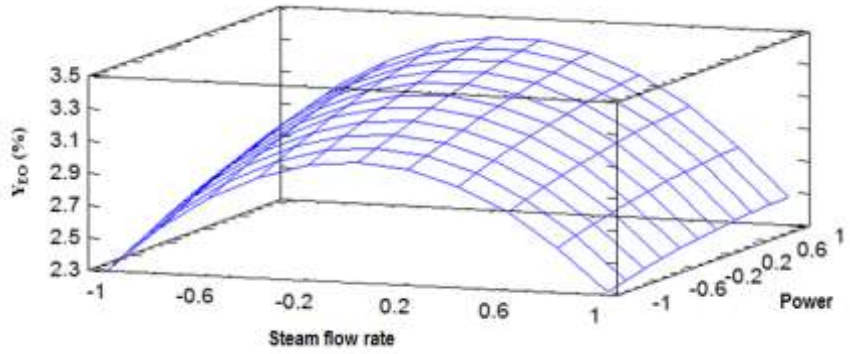


Figure 7. *Response Surface Plots for T. pallescens essential oil depending on the flow-power for t = 6 min^{-1}.*

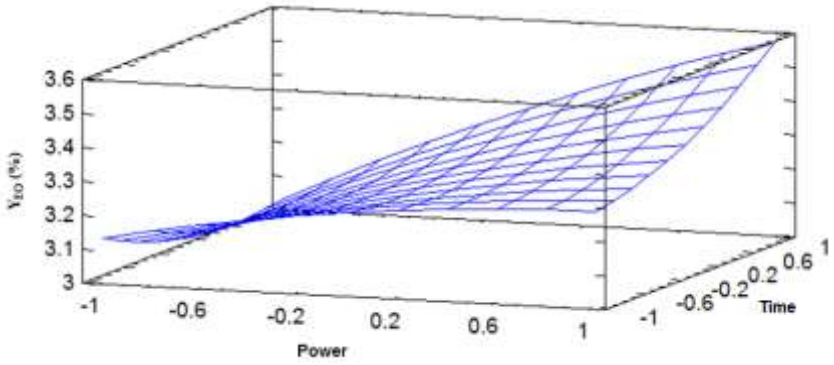


Figure 8. *Response Surface Plots for T. pallescens essential oil depending on the Power-time for Q =10 g.min^{-1}.*

III.4. Insecticidal activity results

Regarding the toxicity tests, the evolution of mortality corrected percentage for the dose of essential oils administered by inhalation and by contact for the two processes (SD) and (MSD) is illustrated by the figures 9 and 10. The results show a significant variation in the bioactivity of the essential oils towards *Rhyzopertha Dominica.*

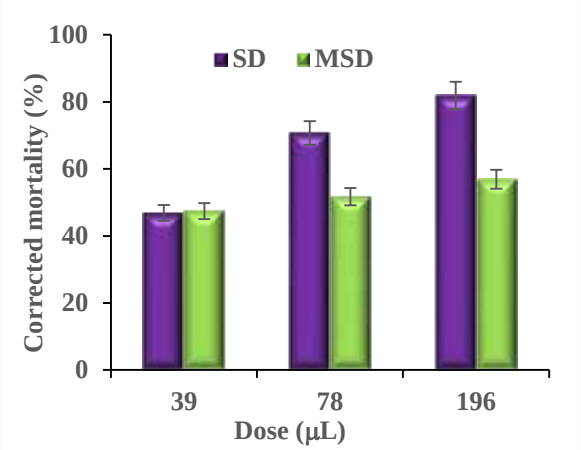


Figure 9. *Percentage of corrected mortality for inhalation toxicity tests as a function of T. pallescens essential oil dose*

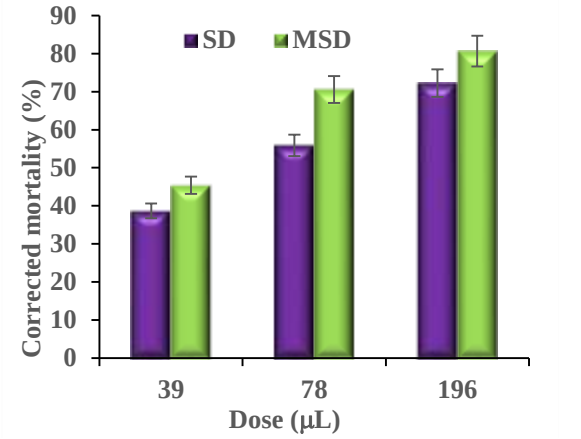


Figure 10. *Percentage of corrected mortality for contact toxicity tests as a function of T. pallescens essential oil dose*

Probit analysis [22] conducted on the corrected mortality shows:

Mortality rate obtained from the essential oil extracted by SD is lower than that extracted by MSD for the contact mode of toxicity for all doses, with a maximum mortality rate of (72.3%) for SD and (80.7%) for MSD, aimed at a dose of 312 (V/V).
In contrast, for the inhalation mode, the mortality recorded by the essential oil extracted by SD is higher than that recorded by MSD for all doses except for 39 V/V dose were the mortalities are equal. The maximum mortality rate is (81.9%) for SD and (56.9%) for MSD, aimed at a dose of 156 (V/V).
The efficiency of the essential oil is also evaluated by LD_{50} for contact mode, and LC_{50} for inhalation mode.
The LD_{50} and LC_{50} values obtained for *T. pallescens* essential oils (table 7) extracted by SD are lower than those obtained by MSD in the inhalation toxicity mode in contrast to the contact toxicity mode where the values obtained by MSD are lower.

These results suggest that *T. pallescens* oils have important insecticidal properties and may be useful as a natural grain protectant against *Rhyzopertha dominica* to replace synthetic insecticides.

Table 7. *Lethal doses of essential oils extracted by SD and MSD to achieve 50% mortality*

Toxicity mode	**Contact LD_{50}**		**Inhalation LC_{50}**	
Prosess	SD	MSD	SD	MSD
C_{EO} (V/V)	41.47	59.12	124.15	85.93

IV. Conclusion

Results of our study show that microwave steam distillation (MSD) is better than steam distillation (SD) in terms of speed: 6 min compared to 15 min for *T. pallescens*, resulting in substantial cost savings in terms of time and energy. The yields obtained by the processes studied are comparable: (3.60; 0.25%) and (3.63; 0.25%) for MSD and SD, respectively and the essential oils are quite similar in composition. The optimal processing conditions obtained for a multivariate study of the MSD process were: 10 g min-1 for steam flow rate, 600 W for microwave power and 6 min for extraction time. These results are in accordance with those obtained from the parametric study. The efficiency of MSD is considerably higher than that of the conventional SD procedure if we consider the short distillation times required without causing changes in the composition of the volatile oil. The essential oils extracted by MSD and SD showed a high toxicity potential against *Rhyzopertha dominica* both by contact and fumigation. This study supports the possibility of using the MSD process as an environmentally friendly alternative method for producing active essential oils.

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