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Research Article

Assessment of yield and chemotypic variation of essential oils from the seed orchard of *Corymbia citriodora*.

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Abstract

As part of *Corymbia citriodora*'s national essential oil project, a seed orchard was set up to support growers' activity. This study aims to assess the essential oil production performance of this orchard. Essential oils extracted by hydrodistillation were analyzed by GC/MS and results were processed by multivariate statistical methods (PCA, HCA) to assess the homogeneity of the future seeds chemotype. The extraction process was modeled by the design of the experiment (DoE) to explore the possibilities of its optimization. The essential oil produced, with maximum yields of 4 %, consisted of citronellal, citronellol and isopulégols, at more than 90 % with a maximum of citronellal content around 80 %. In the laboratory scale, distillation time and hydrodistillation method have positive effects on yield, while the settlement of plant material in the distiller had a negative effect. The citronellal content was less sensitive to factor effects. The *Corymbia citriodora* oil content in Congo ranged from 1.5 to 7.0 %. With a maximum yield of 4 %, the orchard trees produced twice much oil as those of the project stations, and suggest a fast improvement after optimization of sylviculture and extraction. The identified citronella chemotype (citronellal (69-84 %), isopulegol isomers (5-25 %) and citronellol (5-12 %)) met the needs of the market. By current standards, it was an excellent quality oil produced in a low-cost way.

1. Introduction

The research results on *Corymbia citriodora* (ex. *Eucalyptus citriodora*) of Congo-Brazzaville in the last forty years [1], were valorized by a development project on cultivation and distillation *of Corymbia citriodora*, at a domestic level, in rural areas [2]. To support this national initiative on the "Plateau des Cataractes", the Higher School of Technology 'Les Cataractes" in Brazzaville was requested for technical support to the future essential oil producers. So, the Rural Campus of Loukoko (RCL) served as a plantation station for the training of future trainers and producers of the project. The best seedling trees from this orchard will be used to set up clonal plantations by cuttings. The seedling trees will reflect the diversity of the starting plant material. The cloning of the best individuals from the seedling will lead to successful clonal plantations. This orchard will finally be used as an experimental station to set up the project's clonal plantations. The first step of this project was the chemotype identification of the seedling trees of the orchard and the seed distribution to the growers of the best individuals while waiting for the cuttings for the establishment of clonal plantations.

Indeed, three of the four chemotypes highlighted by Penfold and Willis [3], Virmani and Datta [4] for *Corymbia citriodora* were identified in Congo: (i) aldehyde rich chemotype (citronellal), (ii) alcohol rich chemotype (citronellol) and (iii) mixed chemotype (citronellal/citronellol) [1, 5].

This article aims: (i) to determine the chemical composition of the essential oils produced by this orchard in order to identify their chemotype(s) and (ii) to assess influential factors for the optimization of the extraction process used, in order to guarantee a profitable exploitation of the process.

2. Materials and methods

2.1. Site and plant material

A plantation (area: 0.5 ha) of *Corymbia citriodora* was set up at Nkama, a field station of the Rural Campus of Loukoko (RCL), with the technical support of the "Service National de Reforestation (SNR)", station of Kinkala. This plantation, which supplied the training activity in distillation and optimization of equipment and processes with raw materials, will subsequently become a seed and cutting orchard for the establishment of the *Corymbia citriodora* crop in the districts of Boko, Louingui and Loumo, Department of Pool in Congo-Brazzaville.

Nkama station (latitude 4° 26' E, longitude 14° 44', 10 m altitude) was located in Louingui district, 15 km from Kinkala in the south-east, 14 km from Louingui in the north-east and 9 km from the administrative site of the RCL. The site is in "Low-Congo" type climate [6]. The annual rainfall oscillated between 1270 and 1350 mm and the average annual temperature on the "Plateau des Cataractes" varied from 10,5 °C to 23,5 °C.

The site was characterized by sandy soils with 95 % sand, 1% silt, 3.5 % clay; a water pH of 5.3; 2 g/kg of organic carbon and 1% of humus.

The plants of *Corymbia citriodora*, obtained from the SNR (Kinkala station), were planted, in the rainy season, in holes 20 cm in diameter and 30 cm deep with a spacing of 3 m x 1 m on a land weeded with a hoe and cleaned after burning. The mortality rate was relatively high due to the presence of locusts. The establishment of the plantation was therefore spread over 2 years, with the replacement of dead plants, the following season.

Laboratory scale extraction and chromatographic analysis were carried out on leaves collected from trees at least two years old from the seed orchard (NK1 to NK5) and from trees about fifteen years old from the edge of the station (NK6 and NK7), in order to evaluate the extraction yield and the quality of the extracted oils.

2.2. Extraction of essential oils

The essential oils studied were extracted by water hydrodistillation (HD: fully submerged plant material in distillation water) or by vapo-hydrodistillation (VHD: partially submerged plant material) on a Clevenger apparatus from leaves dried in the open air for a week. The essential oil was extracted from the distillate in a separatory funnel and was dried with sodium sulfate. Let m_1 be the mass of leaves and m_2 the mass of the essential oil obtained, the extraction yield is given by:

$$y\ (\%) = (m_2/m_1) \times 100$$

2.3. Determination of the physico-chemical characteristics of essential oils

Relative density, refractive index, acid number, ester number and viscosity were determined according to AFNOR standards [7].

2.4. Chromatographic analysis of essential oils

2.4.1. Gas Chromatography (GC)

The quantitative analysis of the essential oil was performed on an Agilent gas chromatograph, model 6890, equipped with a DB5 column (20m x 0.18mm x 0.18µm). The oven temperature was 50 ° C for 3.2 minutes and then increases to 300 ° C at a rate of 10 ° C per minute, that of the injector is 280 ° C. This device was equipped with a Flame Ionization Detector (FID) hydrogen (40 mL/min) / air (450 mL/min). The flow of the carrier gas (N_2) is 1 mL/minute.

2.4.2. Gas chromatograph /Mass Spectrometry tandem (GC/MS)

The qualitative analysis was carried out using an Agilent gas chromatograph, model 7890 coupled to an Agilent mass spectrophotometer, model 5975, equipped with a DB5 column (20 mx 0.18 mm x 0.18 µm). The oven temperature is 50 °C and remains constant for 3.2 minutes then increases to 300 ° C at the rate of 8 °C per minute, that of the injector is 280 °C. Ionization was done by electronic impact at 70 eV. The m/z mass scan scale ranges from 33 to 450 units. The carrier gas flow rate (helium) was set at 0.9 mL/minute. The identification of the compounds was carried out by comparison of their mass spectra and their retention indices (IR) available in our laboratory bank (http//lexva-analytique.com) and those available in the literature [8, 9].

2.5. Modeling of essential oil extraction

The extraction yield (y) was studied as a function of the duration of the distillation (X_1), the mass of the plant material (X_2) and the method of distillation (X_3):

$y = f(X_1, X_2, X_3)$

with: y: response; f: the response function; X_1, X_2, X_3, the dependent factors. The experiment will therefore consist to assess the factor effects on the response. Ultimately, one has to answer the two following questions: does a factor have a given specific effect on the response and what relationship exists between that factor and the response.

The two-level factorial design as developed by Davies [9] was sufficiently relevant to solve this kind of problem, with low mathematical knowledge [10]. The general formula for the number of experiments (N) for a full factorial design was: N = 2k, with k, the number of variables of the factorial design, if k = 3, $N = 2^k = 2^3 = 8$ experiments.

To build the matrix of experiences, were defined:

a) The reduced variables x_i such that: $x_i = (X_i - X_{io})/\Delta X$, with ($X_{i0}$) = the basic value, value at the center of the experimental domain (level 0) and ΔX: the step of variation = unit of variable variation;

b) The 2 levels of variables: the high level (+1) and the low level (-1).

The domain of study was thus replaced by the domain (-1, +1) and the 8 responses described by the matrix were carried out after randomization. For a first-degree model with interactions, the representative points of a three-variable design of experiments were located in a 3-dimensional space (a cube). The corresponding response function was a first degree polynomial with respect to each of the factors taken independently:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3$$

If the mathematical model associated with the factorial design, was expressed with centered and reduced variables, the coefficients of the polynomial had very simple meanings: mean, a_0; main effects, a_i; interactions a_{ij}, and a_{ijk} [11]. These effects were calculated with JMP SAS software.

2.6. Statistical processing

The descriptive statistics and the graphical representations were carried out on Excel of Microsoft Office Professional Plus 2010, the multivariate statistics (Principal Component Analysis, PCA and Ascending Hierarchical Classification, AHC) on XLSTAT (www.xlstat.com) and the modeling using JMP SAS software.

3. Results

3.1. Adaptation of Corymbia citriodora on the "plateau des Cataractes" (10 years old trees)

Before setting up the seed orchard, the 10 years old trees in Nkama station were studied to assess their adaptation to the local climate and soil.

Table 1 summarizes the chemical composition data of the typical essential oil of these perfectly adapted trees. Out of 45 constituents detected, 42 were identified by their retention indices and their mass spectra. Unidentified Retention Index 1044, 1258, 1259 and 1781 (NI) constituents, which are in trace amounts, making less than 0.55% of the total essential oil. Seven compounds with an individual content greater 1 % led to 93.64 % of the total essential oil.

3.2. Quantitative and qualitative assessment of the essential oil from seed orchard trees (two years old trees)

Table 2 gatherd the extraction yield, the physico-chemical characteristics and the chemical composition of 9 samples studied.

3.2.1. Extraction yield

Extraction yield ranged from 1.28 to 2.59 %. These were low yields knowing that *Corymbia citriodora* yields range from 1.5 to 7.1 % in Congo [5]

3.3.2. Physico-chemical characteristics and chemical profile

The acid number ranged from 1.683 to 2.244. The density, at 26 ° C, which varied from 0.867 to 0.870 g/mL, was lower than that of water (0.996511 g/mL) measured at the same temperature, which led to a variation of the relative density between 0.870 and 0.874. The refractive index, at 26 °C, which varied between 1.4539 and 1.4584, was higher than that of water (1.3356 at the same temperature). The viscosity at 30 °C varied between 1.987 and 2.503 cSt and the ester number between 44.88 and 65.76. The essential oils contained about thirty constituents, with a very large majority, in very small quantities (<1 %) or even in traces (<0.1 %,). Five constituents (citronellal,

Table 1. Composition of essential oil of 15-years -old *Corymbia citriodora* acclimatized to Nkama (CRL)

No.	Constituents	RIc	RIlit	Composition (%)
1.	Isobutyle isobutyrate	911	908	0,03
2.	Alpha pinene	928	932	0,20
3.	Sabinene	968	969	0,04
4.	Béta pinene	972	974	0,48
5.	Myrcene	984	988	0,16
6.	Alpha terpinene	1010	1014	0,03
7.	Para cymene	1019	1020	0,06
8.	Limonene	1023	1024	0,24
9.	1,8-Cineole	1027	1026	1,02
10.	(Z)-bEta-ocimene	1030	1032	0,21
11.	NI	1044		0,06
12.	2,6-Dimethyl-hept-5-enal	1048		0,30
13.	Gamma terpinene	1052	1054	0,30
14.	p-Mentha-3,8-diene	1065	1068	0,33
15.	Terpinolene	1078	1086	0,15
16.	Linalol	1093	1095	0,11
17.	Cis-rose oxyde	1102	1106	0,04
18.	Trans Rose oxyde	1124	1122	0,25
19.	Isopulegol	1149	1145	12,55
20.	Citronellal	1156	1148	65,52
21.	Isoisopulegol	1160	1155	5,92
22.	Néoisopulegol	1172	-	0,84
23.	Neoisoisopulegol	1182	-	0,27
24.	Alpha terpineol	1198	1195	0,11
25.	Nerol	1215	-	0,05
26.	Citronellol	1230	1223	6,44
27.	Neral	1241	1235	0,06
28.	Geraniol	1252	1249	0,27
29.	NI	1258	-	0,07
30.	NI	1259	-	0,06
31.	Géranial	1270	1264	0,07
32.	Citronellic acid	1308	1312	0,24
33.	Para-vinyl-guaiacol	1311	1309	0,03
34.	p-Menthol-3,8-diol cis	1344	1328	1,10
35.	Citronellyle acetate	1350	1350	0,37
36.	Eugenol	1354	1356	0,09
37.	p-Menthol-3,8-diol trans	1366	-	0,38
38.	Beta-Elemene	1393	1389	0,04
39.	Phenylethyle isobutanoate	1395	1393	0,05
40.	Beta caryophyllene	1426	1417	1,09
41.	Alpha humulene	1461	1452	0,06
42.	Germacrene-D	1486	1484	0,06
43.	Elemol	1552	1558	0,04
44.	Caryophyllene oxyde	1588	1582	0,07
45.	NI	1781	-	0,09
	Total			**99,87**

citronellol, iso isopulegol, neo-iso isopulegol and beta-caryophyllene) represented 85-95 % of the total essential oil content (Fig. 1). The Principal Component Analysis, based on the 5 major constituents of the oil, highlighted, through the correlation circle (Fig. 2), a good representation of the variables: citronellal, citronellol, isopulegol, iso-isopulegol and content in essential oil (%); it also indicated that citronellol, was strongly correlated with isopulegol, and citronellal, was correlated with essential oil content. Citronellol was anti correlated with beta-caryophyllene while citronellal was anti correlated with isopulegol and iso isopulegol. This means that, in a given oil, beta-caryophyllene and citronellol increased when the citronellal and the oil contents increased and the amounts of the pulegol isomers decreased.

The distribution of individuals in the plan F1F2 (which contained 71.43% of information) highlighted a very homogeneous class for essential oils from the orchard which excluded 2 individuals (NK6 and NK7, coming from 15-year-old trees); the latter 2 formed a second class characterized, by a low level of citronellal and a high level of isopulegol (Fig. 3).

The Ascending Hierarchical Classification (AHC) confirms the 2 classes suggested by the PCA (Fig. 4, Tables 3 and 4).

Compared to the main constituent: citronellal, the separation value between these 2 classes was 69-70 % [65, 52 % and 57, 26 % (class 2)] < 69 % < [70 -80 % (class 1)]. The extraction process (HD or VHD) did not significantly impact the composition of extracted oils.

In the end, for seven of the 9 studied essential oils of *Corymbia citriodora* acclimatized at the Nkama station, the citronellal content was equal to (or greater than) 69 %. These were very good quality oils, according to their chemical composition (citronellal content) their relative density and their carbonyl number, in addition, to their physical appearance [12]. They were extracted with an average yield of 2 %, which can reach 4 % as it will be shown by following extraction modeling study. This makes it an excellent seed orchard for the development of the essential oil production activity of *Corymbia citriodora* on the "Plateau des Cataractes".

Table 5 gathered the experimental design for factorial design 2^3 for the extraction of *Corymbia citriodora*

Table 2. Physico chemical characteristics and chemical composition of essential oils of *Corymbia citriodora* acclimatized to CRL (Nkama station).

			NK2	NK3	NK4	NK6	NK7	NK2*	NK3*	NK4*	NK5*
	Sample		**NK2**	**NK3**	**NK4**	**NK6**	**NK7**	**NK2***	**NK3***	**NK4***	**NK5***
	Process		**Hydrodistillation**					**Vapo-hydrodistillation***			
Physico-chemical Characteristics	% Essential oil		1.28	2.05	2.75	1.65	2.00	2. 32	2.59	1.97	2.43
	Acid Value						1.683–2.244				
	Specific gravity *						0.867–0.870				
	Relative Density						0.870–0.874				
	refractive index						0.870–0.874				
	Ester Value						44.88–65.76				
	Viscosity **						1.987–2.503				
RIc	**RIlit**	**Constituents**					**Content (%)**				
933	932	α-Pinene	0.07	0.07	0.12	0.2	-	0.06	0.05	-	0.05
971	969	Sabinene	0.22	-	-	0.04	-	-	-	-	-
977	974	β-Pinene	-	0.18	0.53	0.5	0.64	0.15	0.13	-	0.15
986	988	Myrcrne	0.08	0.08	0.07	0.16	-	0.06	0.06	-	0.09
1022	1020	*p*-Cymene	0.05	-	-	0.06	-		0.04	-	-
1027	1024	Limonene	0.09	-	-	0.24	0.24	0.03	0.08	-	0.02
1031	1026	1,8-Cineole	0	0.02	0.04	1.02	0.24	0.07	-	0.05	-
1043	1044	*E*- β-Ocimene	-	-	0.03	0.21	-	-	-	-	0.04
1049	**1047**	Berganal	0.26	0.1	0.14	-	-	0.12	0.17	0.45	0.32
1056	1054	γ-Terpinene	0	0	0	0.3	0.23	0		-	0
1068	1068	Mentha-3,8-diene	0.11	0	0.08	0.33	0.53	0.12	0.08	-	0.1
1083	1086	Terpinolene	0.06	-	0.06	0.15	0.31	0.08	-	-	-
1096	1095	Linalol	0.21	-	0.34	0.11	0.27	0.16	-	0.27	0.2
1124	1122	*trans* Rose Oxyde	-	-	-	0.25	-	-	0	0.36	-
	1445	Isopulegol	tr	tr	tr	**12.55**	**16.04**	tr	tr	tr	tr
1159	1148	Citronellal	74.35	79.41	80.38	**65.52**	**57.26**	75.99	78.83	69.41	78.34
1166	1155	Iso Isopulegol	6.77	4.78	4.5	5.92	8.83	6.3	4.62	6.58	6.63
1175	1167	Néoiso Isopulegol	1.11	0.67	0.64	0.27	1.12	1.13	0.77	0.95	-
1179	-	NI	0.24	0.14	0.15	-	-	0.23	0.14	0.19	1.11
1198	1200	γ-Terpineol	0.05	0.1	-	-	-	0.04	-	-	0.2
1229	1223	Citronellol	11.59	8.92	7.56	5.44	6.43	9.64	7.65	10.98	7.37
1312	1312	Citronellic acid	0.12	0.79	0.43	0.24	1.03	0.51	0.5	1.87	0.16
1347	1350	Citronellyl Acetate	0.14	0.62	0.45	-	-	0.14	0.46	0.74	0.21
1355	1356	Eugenol	0.16	0.05	0.1	-	-	0.23	0.04	0.13	-
1395	-	NI	0.29	0.25	0.29	-	-	0.35	0.3	0.41	0.2
1429	1417	β- Caryophyllene	0.49	1.12	1.68	1.09	2.46	1.59	2.14	2.72	2.18
1434	1434	γ-Elemene	-	0.03	0.04	-	-	0.05	0.06	0.05	0.03
1465	1452	α-Humulene	-	0.07		-	-	0.1	-	0.17	0.12
1490	1484	Germacrene-D		0.09	0.1	0.06	0.21	0.16	0.19	0.07	0.06
1504	1500	Bicyclogermacrene	-	0.09	0.07	-	-	0.12	0.13	0.08	0.03
1524	1522	δ-Cadinene	-	0.04	0.04	-	-	0.01	0.07	0.07	-
1588	1576	Spathulenol	0.07	0.09	0.03	-	-	-	0.07	0.1	0.03
1593		Caryophyllene Oxyde	0.18	0.15	0.22	0.07	-	0.15	0.26	1.11	0.22
		Total	96.70	97.9	98.09	94.73	95.84	97.59	96.80	96.80	97.90

* Specific gravity (pycnometer: 26 °C, g/mL); ** viscosity (Oswald viscometer : 26 °C, cSt); NI : non identified ; tr : traces

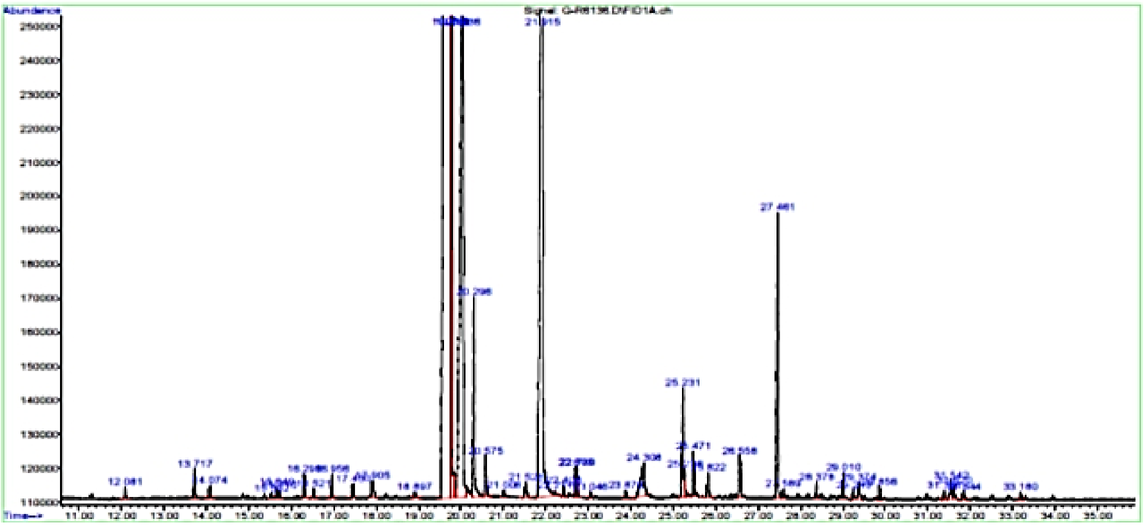

Figure 1. Chromatogram of essential oil of 15-years older *Corymbia citriodora* acclimatized to Nkama (CRL)

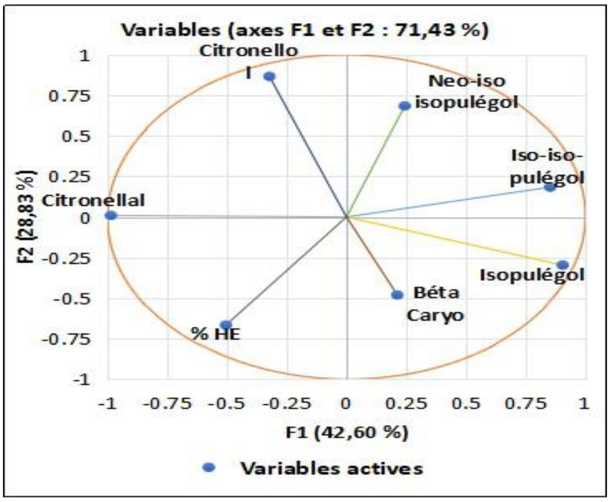

Figure 2. PCA correlation circle for essential oils from *Corymbia Citriodora* acclimatized to Nkama (RCL)

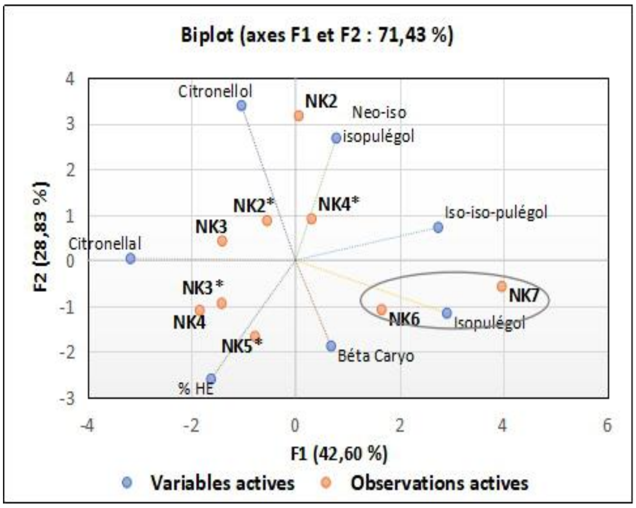

Figure 3. Distribution of individuals in PCA on the first main plan F1F2 (71.43 % of information)

Table 3. Distribution into 2 classes by AHC of esential oils *C. citriodora* acclimatized in Nkama (CRL)

Classes	1	2
Objets	7	2
	NK2*	NK6
	NK2	NK7
	NK3*	
	NK3	
	NK4*	
	NK4	
	NK5*	

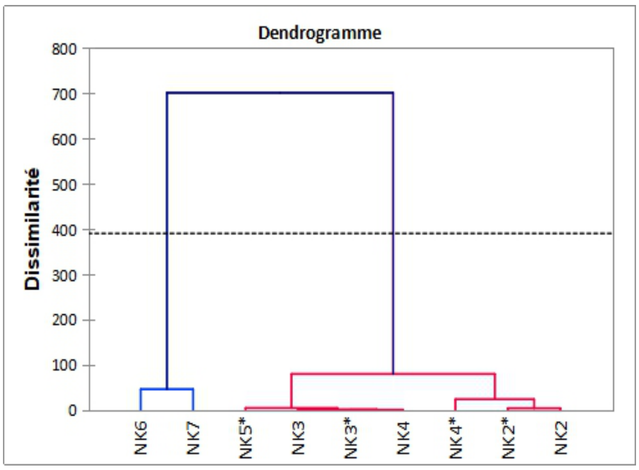

Figure 4. Distribution in 2 classes by AHC of essential oils *Corymbia citriodora* acclimatized at Nkama RCL (dendrogram)

essential oil in the laboratory scale, with respectively: X_1, the duration of the distillation, X_2, the mass of plant material and X_3, the distillation method. Table 6 gathered the responses (% HE, % citronellal, %

Table 4. Composition of central objects of the 2 classes (A et B) of essential oils *Corymbia citriodora* acclimatized to Nkama (CRL)

Classes	Citronellal	Isopulégol	Iso-iso-pulégol	Néo-iso isopulégol	Citronellol	Béta Caryophyllène	% HE
1 (NK2*)	75,99	0,00	6,30	1,13	9,62	1,59	2,32
2 (NK6)	65,52	12,55	5,92	0,27	5,44	1,09	1,65

Table 5. Design of experiments for the extrac-tion of essential oils *Corymbia citriodora* acclima-tized to CRL (Nkama station)

Run	t (h) : X_1	Mass (g) : X_2	Method : X_3
1	1,5	118	HD
2	3	118	HD
3	1,5	220	HD
4	3	220	HD
5	1,5	118	VHD
6	3	118	VHD
7	1,5	220	VHD
8	3	220	VHD

HD: Hydrodistillation; VHD: Vapo hydrodis-tillation

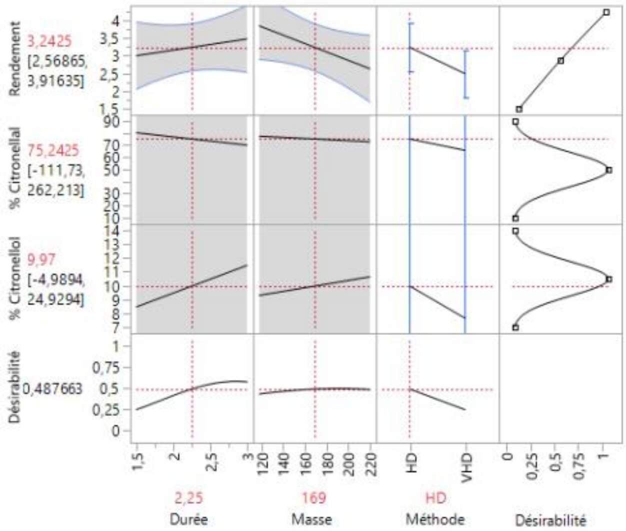

Figure 5. Effects of factors and desirability on the extraction of *Corymbia citriodora* essential oil. (Meaning of French terms in the figure.: rendement : yield; désirabité : desirability; durée : time; masse : mass; méthode : method)

Table 6. Oil content and chemical composition of oils extracted *via* the design of experiments

Run	1	2	3	4	5	6	7	8
Méthod	HD	HD	HD	HD	VHD	VHD	VHD	VHD
% Essential oil	**3.60**	**4.10**	**2.41**	**2.86**	**2.71**	**3.14**	**1.72**	**2.40**
α-Pinene	0.00	0.05	0.04	0.00	0.00	0.05	0.09	0.10
β-Pinene	0.08	0.00	0.12	0.00	0.09	0.13	0.00	0.20
Myrcene	0.00	0.09	0.00	0.00	0.05	0.09	0.11	0.10
Limonene	0.05	0.00	0.00	0.00	0.31	0.07	0.08	0.00
1,8-Cineole	0.35	0.29	0.31	0.00	0.04	0.31	0.32	0.00
Berganal	0.3	0.14	0.16	0.7	0.21	0.14	0.14	0.30
Linalol	0.31	0.3	0.26	0.39	0.26	0.28	0.26	0.20
Citronellal	**77.00**	**78.09**	**83.66**	**62.22**	**84.10**	**83.13**	**78.26**	**78.00**
Iso isopulegol	**6.36**	**6.2**	**5.01**	**7.76**	**4.4**	**4.66**	**6.17**	**6.6**
Neo iso Isopulegol	0.00	0.88	0.49	1.03	0.29	0.41	0.81	0.00
γ -Terpineol	0.06	0.08	0.05	0.95	0.04	0.05	0.05	0.20
Citronellol	**9.27**	**9.33**	**7.67**	**13.61**	**7.49**	**7.11**	**8.56**	**7.40**
Citronellic acid	0.86	0.51	0.37	2.43	0.66	0.58	0.49	0.20
Citronellyle acetate	0.27	0.09	0.09	0.64	0.07	0.08	0.15	0.20
Eugenol	0.00	0.04	0.07	0.26	0.05	0.06	0.11	0.00
β-Caryophyllene	0.11	0.5	0.32	0.7	0.37	0.84	1.57	2.20
α-Humulene	0.00	0.04	0.00	0.00	0.00	0.06	0.1	0.10
Germacrene-D	0.00	0.04	0.00	0.00	0.00	0.09	0.12	0.10
Bicyclogermacrene	0.00	0.05	0.00	0.00	0.00	0.1	0.12	0.00
Spathulenol	0.05	0.1	0.05	0.39	0.05	0.07	0.06	0.00
Caryophyllene oxyde	0.06	0.12	0.06	1.06	0.08	0.09	0.11	0.20
Total	**95.13**	**96.94**	**98.73**	**92.14**	**98.56**	**98.4**	**97.68**	**96.10**

citronellol) generated by the design of experiments used. The processing of the design using JPM SAS software for the extraction led to the following mathematical model:

$y = 2.90 + 0.26x_1 - 0.52x_2 - 0.38x_3 + 0.03x_1x_2 - 0.02x_1x_3 - 0.09x_2x_3$

The main factors effects were reported in Table 7 and this study established that: (i) the average yield of extraction was 2.90 %; (ii) the main effects of the 3 factors were of the same order of magnitude with one positive (a_1) and two negatives (a_2 and a_3): The extension of the extraction time and moving from vapo-hydrodistillation (VHD) to hydrodistill-ation (HD) improved the extraction yield, while the compaction of the plant material, which had the most important factor effect, increased this yield; (iii) the interaction effects were much weaker than those of the factors; they can therefore be neglected. The model equation, especially with a domestic process, can be reduced to:

$y = 2.90 + 0.26x_1 - 0.52x_2 - 0.38x_3$\

Table 7. Effect of factors and interactions on the extraction of *Corymbia citriodora* essential oil

Terms	Effect of factor Estima-tion	Standard Error	t ratio	Prob. > |t|
Constant (a_0)	2.8675	0.0375	76.47	**0.0083**
Time (a_1)	0.2575	0.0375	6.87	**0.0921**
Mass(a_2)	-0.52	0.0375	-13.87	**0.0458**
Method(a3)	-0.375	0.0375	10.00	**0.0635**
Time *Mass (a_1a_2)	0.025	0.0375	0.67	0.6257
Time *Method (a_1a_3)	-0.02	0.0375	-0.53	0.6881
Mass*Method (a_2a_3)	-0.0875	0.0375	-2.33	0.2578

Fig. 5 summarized all the factors effects on the citronellal and citronellol contents and the expected results under optimal conditions. They were either slightly all tend towards 0 (x-axis). They were therefore not significant within the chosen domain of study. However, one can hope to extract essential oil with a yield of 3.25 % containing approximately 75 % of citronellal and 10 % of citronellol starting from 169 g of plant material for 2 h 15 min of distillation time and by hydrodistillation method (desirability).

4. Discussion

The two-years-old trees in Nkama orchard produce 1.3-4.1 % essential oils. This production is higher than those observed on the operational sites of the Eucalyptus project (Pointe Noire, Loudima, PK 45, Gamboma, Kinzomo (DR Congo), which is on average 1.5 %. Trees acclimatized in Congo-Brazzaville kept the yields of their Australian parents [14713 (S and W of Mt Garnet OLD], introduced in Congo in 1989 [1, 2, 13]. This yield observed over ten years for a hundred trees acclimatized in Congo-Brazzaville which led to 2.0 - 7.0 % of essential oil content [1, 5, 13], suggested quantitative improvement by optimization of cultivation techniques and/or extraction processes. These last yield levels were consistent with those reported in the literature (Table 8), in particular in Benin [14], Burundi [15], Nigeria [4], Kenya [16], Cuba [17], Chile [18] and India [19]. The physical aspect, the relative density, the refractive index and the carbonyl index of the oils extracted from the trees of the Nkama seed orchard comply with AFNOR Standards [7].

Table 8. Physico-chemical characteristics of *Corymbia citriodora* essential acclimatized in Congo.

Physico-chemical parameters	Nkama Orchard	Planta-tion UR2PI (PNR)*	Eucaly-ptus Project	AFNOR Stand-ards
Acid Value (mg KOH/mL)	1.68–2.24	1.12 – 3.65	-	-
Ester Value	44.88–65.76	-	-	-
Specific Gravity g/mL (20°C)	0.867–0.870	0.854 – 0.889	0.866 – 0.875	0.860 – 0.870
Refractive index n	1.4539–1.4584	-	1.4546-1.4585	1.450-1.459
Rotative power α	-	-	-0.96 à 1.84 °	–1 à 3°

*Research Unit on Industrial Plantation Productivity (Pointe Noire)

They are also of the same order of magnitude as those of the oils produced by the experimental plantations of UR2PI in Pointe Noire or by the eucalyptus project in Pointe Noire, Loudima, PK45, Gamboma and Zomono (Table 9). The "type" variety, as defined by

Penfold and Willis [2] and Boland et al. [20] was the one most often found throughout the world, with more than 65 % citronellal [14, 18, 21, 22]. And the Congo was not an exception. However, Chalchat et al.

Table 9. Composition in major constituents of *Corymbia citriodora* essential oils of different origins.

Origin	Citro-nellal (%)	Citro-nellol (%)	Isopul-egols (%)	Autres (%)	Refere-nces
Congo	78.25	4.78	5.10	-	[24]
Mali	76.33	7.25	-	Methyl eugenol (8.21%)	[25]
Brésil	61.78	7.90	15.54	-	[26]
Bénin	52.80	20.0	7.8	citronellyle acetate (9 %)	[27]
Inde	52.20	12.3	11.9	-	[28]
Inde	70.30	8.8	-	β-caryophyllène(2.6 %) citronellyle acetate (1.3 %)	[29]
Inde	69 - 87	5- 10	0.9 – 3.1	Linalool (2.1–6.4 %) citronellyle acetate (0.4 – 1.2)	[19]
Algérie	69.77	10.63	4.66	-	[30]

[23] observed more than 55% of 1,8-cineole in essential oil of *E. citriodora* from Rwanda (Table 9). The essential oils extracted from the two-year-old trees of the Nkama seed orchard contain 69-84 % of citronellal, 5-25 % of isopulegol isomers and 5-12 % of citronellol. They belong to the main aldehyde chemotype, which, very generally, is citronellal, and with a content greater than 70 %. Some essential oils of *Corymbia citriodora* acclimatized in Congo reached 90 % of citronellal content [1,13] (Silou *et al.*, 2013, 2019). Compared to those of the four project sites, the oils from the Nkama seed orchard seem more interesting with 80 % citronellal against 57.01 % in Pointe Noire, 72.79 % at PK45, 62.36 % in Gamboma and 72, 07 % in Dzomono (DR Congo).

Modeling of the extraction, in the laboratory scale using a full three-factor factorial design, demonstrated the superiority of hydrodistillation to vapo-hydrodistillation over the extraction yield, while the settling of plant material in the distiller decreased the extraction yield. In addition, extending the distillation time increased extraction yield, but with no effect on the citronellal content. In the end, you had more essential oil of very good quality.

5. Conclusions

Corymbia citriodora trees of the orchard set up in Congo, to support the participants in the Eucalyptus project, led to very good quality essential oils with yields varying from 1 to 4 %. The first main constituents make up over 90 % of the total essential oil, with following chemotype: % citronellal > % citronellol > % isopulegol ; citronellal content was up to 80 %. The extraction process was modeled using a full factorial design 2^3 to explore the possibilities of its optimization. In the laboratory scale, the distillation time and hydrodistillation method had positive effects on the yield while the compaction of the plant material still had a negative effect. The citronellal content was not sensitive to the factors effects. A yield of 3.25 % with a citronellal content of 75 % was expected from the hydrodistillation of 160 g of *Corymbia citriodora* leaves for 2 h 15 min. By current standards, Nkama ochard led to an excellent quality oil, produced in a very profitable way for industrial purposes.

Authors' contributions

Conceptualization, validation and writing: Thomas Silou; technical supervision: Bassiloua Jean Bruno, Investigation: Praudyge Jaynereuse Nombault Nienzy and Andeouene Baou.

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Conflicts of interest

No conflict of interest

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