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Pilot Scale Modeling Of Biomass And Essential Oil Production From *Cymbopogon Nardus* (L.) Rendle, Acclimatized On The "Plateau Des Cataractes" In Congo-Brazzaville

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Abstract

Cymbopogon nardus (L.) Rendle, acclimatized in Congo-Brazzaville was planted on an experimental field for modeling the leaf biomass production and essential oil extraction using complete factorial design 2^3. During 12 months, the best results were expected at 8.5 months for a planting spacing of 37.5 cm, with an average production of 4.2 t / ha of leaf biomass, 12 offshoots / plant (tall or foot) and 53 leaves / offshoot. The essential oil production was not very sensitive to the harvest time in the day, but significantly sensitive to the packing of the plant material in the distiller and the duration of the distillation. From the experimental conditions selected for this study, the best results expected was an average yield of 2 % from 12.5 kg of plant material harvested at 12:00 o' clock, dried for a week and distilled for 4 hours. The essential oils extracted from the starting offshoots and the new offshoots collected on experimental field, was found of the same chemotype, characterized by the same 3 first major constituents (citronellal (44,0 ± 0,9) geraniol (22,7 ± 1,1) citronellol (10.9 ± 0.6)) accounting for more than 75 % of the total composition of the essential oil.

1. Introduction

The genus Cymbopogon, which belongs to the Poaceae family gathers about 140 species including many aromatic plants and growing throughout the tropical zone of the five continents. More than fifty-two have been reported to occur in Africa, forty five in India, six in Australia, six in South America, four in Europe, two in North America and the remaining in South Asia [1]. Among these 140 species, sometimes having several varieties per species, ten, widely distributed, produce essential oils of world importance. Cymbopogon species display wide morphological and essential oil composition variations. [2]. Despite this wide biodiversity, only two oil chemical profiles lead to the two main groups of aromatic species of this genus: (i) "lemongrass" group, which produces lemon scented essential oils mainly consisting of citral, includes *Cymbopogon citratus* (west indian lemongrass), *Cymbopogon flexuosus* (east indian lemongrass, cochingrass or malabargrass) and *Cymbopogon pendulus* ; (ii) "citronella" group gives essential oils mainly consisting of geraniol, citronellal and citronellol, in small quantities. Geraniol is responsible for their pink smell; those are *Cymbopogon nardus* and *Cymbopogon winterianus*, two very similar species which some authors consider as two varieties of the same species *C. nardus var.* Java and *var.* Ceylan [1, 2, 3, 4, 5]. A citronellal chemotype of *Cymbopogon flexuosus* were reported in Bengladesh [6] and in Congo Brazzaville [7]. The essential oils of *C. nardus* and/or *C. winterianus* have been very well studied in Asia and South America, for their chemical composition [1-4, 8 - 10], for their biological properties [7-16]; and for their extraction mechanism [16, 17]. These studies are less abundant in sub-Saharan Africa [4, 7, 18-20].

For three last decades the Republic of Congo had strongly supported the development of a rural essential oil production crop. Indeed, after setting up some executive infrastructures: the Non-timber Forest Products Valorization Center, and the National Reforestation Office, the Government of the Republic of Congo implemented between 2010 and 2015 a project entitled "Development of marketing channels for high value-added *Eucalyptus citriodora* essential oils by village communities in Congo (Project CFC / ITTO / 80 / PD 364/05 Rev.4 (I))", giving a great opportunity to the valorization of the research results of *Eucalyptus citriodora* conducted in Congo for at least two decades [21]. In addition, and in order to join this major national initiative, local populations from the Louingui and Loumo districts on the "plateau des Cataractes" have chosen the development of the lemongrass and citronella essential oils and the Higher School of Technology of "Cataractes" in Brazzaville has been requested for scientific and technical support. So, we followed in this context, for more than ten years, the acclimatization of *Cymbopogon nardus* (L.) Rendle, *Cymbopogon citratus* L. (DC) Stapf and *Cymbopogon flexuosus* (Nees ex Steud.) Wats on the "plateau des Cataractes" in Congo-Brazzaville (Congo Basin) to certify the chemical composition of the essential oils produced by local producers. Some relevant results have been obtained at the laboratory scale [21-23]. But the gap between laboratory and pilot scale requiring much more logistical and financial inputs, we avoided the multiplication of experiments to optimize biomass production and improve essential oil quality by using mathematical models to predict results of optimized processes. We report here results obtained in the modeling of the biomass production and the essential oil extraction in support of essential oil producers from the "plateau des Cataractes" working in the Project CFC / ITTO / 80 / PD 364/05 Rev.4 (I)) and in "plateau des Cataractes Citronella initiative".

2. Material and Methods

2.1. Experimental culture site of Nkama

This study was carried out in Congo-Brazzaville, on the "plateau des Cataractes" at Nkama, in Louingui district (latitude 4 ° 26 'E, longitude 14 ° 44', 10 m altitude). The site is located 15 km from Kinkala, chief-town of the Department of Pool, and 14 km from Louingui, chief-town of the district and 9 km from the administrative site of the Rural Campus of Loukoko (Higher School of Technology of "Cataractes") and is subject to the "low-congolese climate" type [24]. The annual rainfall varies between 1270 and 1350 mm, the annual average temperature on the "plateau des Cataractes" can oscillate between 10.5 ° C to 23.5 ° C. The site is characterized by sandy soils with 95% sand, 1% silt, 3.5% clay, pH 5.3; 2g / kg of organic carbon and humus of the order of 1%. The field was set up after a fallow period with a recent history of pigeon pea cultivation for animal feed.

2.1.1. Soil preparation

Manually soil cropping for the experimental field began in October 2017 with hoe weeding. In November, the stirred soil was loose, aerated and had been worked on about 15 cm deep, manually, using the hoe. On two planted areas called block1 (B1) and block 2 (B2) were installed boards : 15 cm high, 3 m wide with length varying between 16 m (B1) and 24 m (B2), separated by 50 cm circulation corridor (Figure 1a). Three planted areas called block 3 (B3), block 4 (B4) and block 5 (B5) were planted directly on the ground (no boards) after weeding, burning and cleaning while respecting the board organization of the planted field (3 planted rows separated by a 50 cm circulation corridor, Figure 1b).

(a) Planting with boards

(b) Planting without boards

Figure 1. Experimental plantation of *Cymbopogon nardus* at Nkama

2.1.2. Plant material

Three species of the *Cymbopogon genus* (*C. citratus*, *C. nardus*, *C. flexuosus*) and one species of the Ocimum genus (*Ocimum basilicum*) were planted at a pilot scale in the experimental field of the Higher School of Technology of "Cataractes" to Nkama, on a valley with a narrow slope in which flows a small waterway . This work, concerns *C. nardus* planted on boards (B2) and on unworked soil (B3) (Figure 2). Native of India and Southeast Asia, *Cymbopogon nardus* is a tropical plant, widely acclimatized in Africa, South America, Central America and Europe. *C. nardus* is a perennial herb that grows to a tuft height of 1.5 m, starting from stems; purple in color, its leaves are narrow and lanceolate; the inflorescences appear one year after planting. The starting offshoots used were introduced in Congo by the Higher School of Technology of "Cataractes" from Benin. After acclimatization, this raw plant material was distributed in the districts of Louingui and Loumo in Congo-Brazzaville [23].

Figure 2. *Cymbopogon nardus* acclimatized in the experimental field in Nkama

2.1.3. Offshoots planting

The plantation of *C. nardus* offshoots was done on 2017, December 28th and 2018, March 29th, with 30 to 40 cm offshoots, without leaves.

(i) A first group of *Cymbopogon nardus* offshoots (3207plants) were planted on 18 boards, on an area of 507 m^2 (0.05 ha) on Block 2 (B2) with 3 planting spacings (Figure 1a):

- ✓ 25 cm spacing : 8 boards with a total area of 217.6 m^2, at a rate of 6 plants / m^2, (60 000 plants / ha)
- ✓ 50 cm spacing : 8 boards with a total area of 217.6 m^2, at a rate of 3 plants / m^2 (30 000 plants / ha)

✓ 40 cm spacing : 2 control planks, 17.2 m2 area with a density of 4 plants / m^2 (40 000 plants / ha). Eight (8) of the 18 boards constitute a complete factorial plan 2^3: 4 boards, 25 x 25 cm; 4 boards, 50 x 50 cm.

(ii) A second group of offshoots were planted in B3, with no special soil work in holes 15 cm in diameter and 15 cm deep, leading to a block of 20 elementary plots (3 rows of plants separated by 50cm circulation corridors) with 2 planting spacings : 25 cm for 8 plots at a density of 6 plants / m^2 and 50 cm for 12 elementary plots at a density of 4 plants / m^2. There are, in total, 4488 plants in B3 (figure 1b).

2.1..4. Follow-up of the experimental field

Weeding was carried out every two months, if necessary. The plots were not irrigated in the dry season therefore the plants were under heavy water stress. Crop evolution was followed at well-defined time intervals by the determination: (i) of the number of leaves per offshoot, and the number of offshoots per plant (ii) the elongation of the leaves and (iii) the aerial biomass by weighing, using a balance. Random samples were done for each studied block (B2 and B3):

(i) Block 2 : 3 boards planted 25 cm spacing and 3 plants drawn randomly on each board and 3 boards planted 50 cm spacing and 3 plants drawn randomly on each board.

(ii) Block 3: 3 elementary plots planted 25 cm spacing and 3 plants drawn randomly on each elementary plot and 3 elementary plots planted 50 cm spacing and 3 plants drawn randomly on each plot. This operation was carried out every 3 months (4 samplings per year).

2.2. Essential oil extraction

A pilot extraction was carried out in 250 L distillers [4, 23]. The plant material and distillation water, placed in the same reactor (0.5 m×0.5 m×1 m), were not separated by a removable perforated steel partition. The condenser was a pipe of inside diameter 2 cm, fitted with an expansion chamber, running through a tank (0.5 m× 0.5 m×1 m), in which water flowed in the opposite direction to the distillate. The maximum load of plant material was 30 kg. The essential oil, collected by decantation, was dried with sodium sulphate.

The yield of the extraction is given by the relation:

$$\text{Essential oil content (\%)} = 100\,\frac{m_1 - m_2}{m_1}$$

with m_1 = mass of the sample (plant material) and m_2 = mass of the essential oil obtained.

2.3. Chromatographic analyzes

2.3.1. Gas Chromatography (GC)

GC analysis was performed on an Agilent 6890 instrument equipped with a split/splitless injector (280 °C, split ratio 1 : 10), using a DB-5 column (30 m×0.25 mm, df: 0.25 µm). The temperature was ramped from 50°C (5 min) to 300°C at 5°C/min. Injector and detector temperature was 280°C. Helium was used as carrier gas at a flow rate of 1 mL/min. The sample material consisted of 1.0 L of oil diluted to 0.05% (v/v) with acetone.

2.3.2. Gas Chromatography(GC) / Mass Spectrometry (SM)

GC/MS was performed on an Agilent 5973/6890 system operating in EI mode (70 eV), equipped with a split/splitless injector (280 °C, split ratio 1 : 20), using a DB-5 column (30 m×0.25 mm, df: 0.25 µm). The temperature was ramped from 50°C (5 min) to 300 °C at 5°C/min. Injector and detector temperature was 280°C. Helium was used as carrier gas at a flow rate of 1 mL/min. The compounds were identified by comparing their mass spectra and retention indices (RI) with those of the Adams [25], NIST [26], König *et al*. [27] and those of the laboratory LEXVA analytic.

2.4. Techniques for modeling biomass production and essential oil extraction

4. 1. Experiment design for modeling biomass production

To evaluate the effects of <plant position on the valley >, <planting spacing>, and <harvest month>, we used a complete factorial design 2^3, [2] [28]. Biomass production (y) was studied according to the position on the valley (X_1) of the planting spacing (X_2) and the age of the plant (X_3). This lead mathematically to : y = f (X_1, X_2, X_3) with: y: the response; f: the response function; X_1, X_2, X_3 the factors. The experiment will therefore consist in highlighting the effects of certain factors on the response. In the end, one must answer the following double question: does a factor have a specific effect on the response and what is the relationship between this factor and the response? The two-level factorial design as developed by Davies [29] seems sufficiently adapted to solve this type of problem and it has the advantage of appealing only to very elementary mathematical knowledges [30]. The general formula of the number (N) of experiments for a complete factorial design is: $N = 2^k$, with k, the number of variables of the factorial design. If k = 3, $N = 2^K = 2^3 = 8$ experiments to build the experiment matrix, we define reduced variables xi such that:

$$xi = (Xi - Xi_0) / \Delta X$$

with: (Xi_0) = the base value, value at the center of the experimental domain (level 0),
ΔX: the step of variation = the unit of variation of the variables;
2 levels of variables: the high level (+1) and the low level (-1).

The field of study is thus replaced by the domain (-1, +1) and the 8 responses described by the matrix are performed after randomization. For a first-degree model with interactions, the representative points of a three-variable experiment design are located in a 3-dimensional space (a cube). The corresponding response function is a polynomial of the first degree thanks to each factor taken independently, noted:

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

Since the mathematical model associated with the factorial design is established with the centered and reduced variables, the coefficients of the polynomial then have a very simple meaning: mean, a_0; main effects, a_i; interactions a_{ij}, and a_{ijk} [28]. These effects were calculated with the JMP SAS software.

2.5. Design of Experiment for essential oil extraction

A first-degree model complete factorial design, with 3 factors, and their interactions noted:

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

with the centered and reduced variables, y leads to the following polynomial

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

The main factor effects (a_i) and the interactions between factors (a_{ij}, a_{ijk}) are obtained using the JMP SAS software with the levels defined in Table 1 and the matrix of experiments given in Table 2.

Table 1. Niveaux de variation du plan d'expériences

Levels	X_1: Harvest period	X_2 : Mass (kg)	X_3: Extraction duration (h)
Low (-1)	8-9 h	10	2
High (+)	15-16 h	25	6

Table 2. Expriment matrix of a factoriel complet design with 3 factors

Experiment run	X_1	X_2	X_3
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1

3. Results and discussion

3.1. Evolution of biomass production in the experimental field

Table 3 gives an overview of the results, over 12 months: the growth of the plant (height), leaves (length, width) and the biomass production (number of offshoots per plant and number of leaves per offshoot) of the studied plant material : *C. nardus* planted on blocks 2 and 3. For the two blocks and for the two cropping conditions, the height of the plant is correlate to the size of the longest leaf :

$$(\text{offshoot height}) = 1.1625\ (\text{leaf length}) + 9.872 \text{ with } R^2 = 0.92$$

With a coefficient of determination $R^2 = 0.92$, the correlation is very good.

During the first 3 months, all studied leaves reached 2 cm wide by a total of 2.5 cm for leaves that became adults from 8 months (Figure 3). No linear correlation could be found between length and width when growing a *C. nardus* leaf from the Nkama experimental field.

Table 3. Growth and production data of *C. nardus* acclimatized at Nkama

Board Code*	Offshoot height (cm)	Leaf length (cm)	Leaf width (cm)	Offshoot Number/plant	Leaf Number/offshoot
***C. nardus* planted in cropped soil (boards:B2)**					
Planting spacing : 50 cm					
CN 3/50/B2	89.7 ±11.5	71.0±6.0	2.0±0.0	4±0	18±4
CN 5/50/B2	128.7±9.4	108.9±13.0	2.2±0.0	7±3	53±23
CN 8/50/B2	132.4±11.4	100.3±8.0	2.4±0.1	21±7	92±24
CN 12/50/B2	119.8±23.2	89.2±14	2.5±0.1	21±4	115±24
Planting spacing : 25 cm					
CN3/25/B2	89.7±11.5	71.0±6.0	2.0±0.0	4±1	18±4
CN5/25/B2	109.3±0.0	80.7±0.0	2.3±0.0	6±0	23±0
CN8/25/B2	125.6±11.0	97.6±10.0	2.5±0.2	15±2	64±9
CN12/25/B2	119.4±25.0	89.6±8.8	2.5±0.1	15±4	78±28
***C. nardus* planted in uncropped soil (B3)**					
Planting spacing : 50 cm					
CN 5/50/B3	90.8±5.0	72.1±4.0	2.5±0.0	7±3	43±19
CN 8/50/B3	107.8±6.7	86.2±2.5	2.3±0.3	14±6	66±26
CN 12/50/B3	96.7±2.8	74.0±4.4	2.2±0.1	18±2	95±10
Planting spacing : 25 cm					
CN5/25/B3	91.16±2.2	71.53±1.5	2.2±0.1	5±0	24±2
CN8/25/B3	94.4±1.6	73.0±1.8	2.1±0.0	7±1	30±2
CN12/25/B3	107.4±4.3	83.7±3.2	2.3±0.2	13±1	71±10

* CN 3/50/B3 : *C.nardus*/ harvested in march (3) / spacing plantation : 50 cm/ uncropped soil: B3.

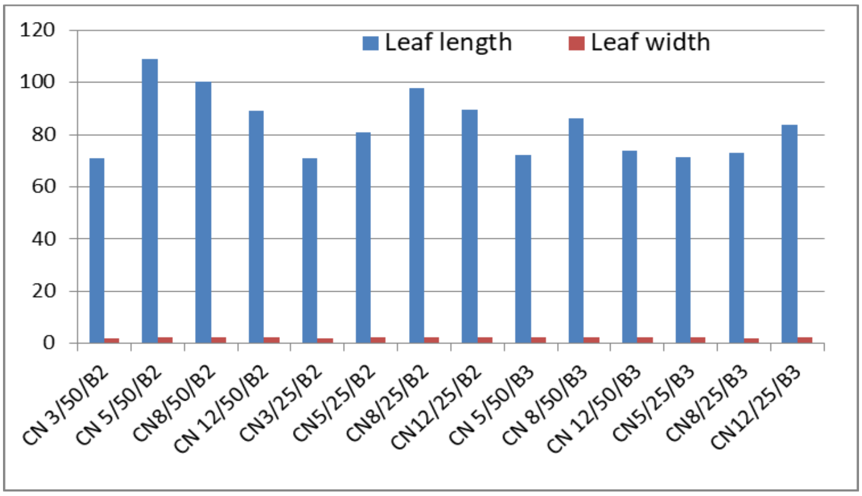

Figure 3. Data <length– width> in *C. nardus* leaves growing in experimental field at Nkama

A more detailed examination of the results indicates that:

(i) Whatever soil preparation or planting spacing, the curve representing the growth of the plant and that of the leaf are synchronous: their maxima and minima are superimposed (Figure 4).

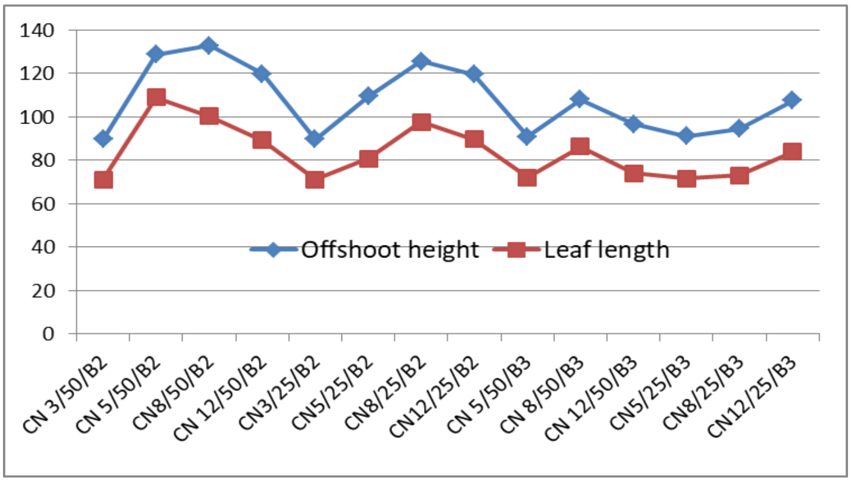

Figure 4. Growth of plants and leaves as fonction of cropping conditions of *C. nardus* in the experimental field of Nkama

(ii) During one year, and for a crop on board, the size of the plant and the leaves increases, passing through a maximum in May (50 cm spacing) and in August (25 cm spacing) and decreases then after. The increase in planting spacing accelerates the growth of the plant and leaves. This is probably due to a greater availability of nutrients in crop with large planting spacing. This growth is also faster, then the maximum is reached earlier (May) for the 50 cm spacing than for the 25 cm spacing (August).

(iii) On block 3 (uncropped soil), one observes similar behaviors with smaller variation range and a resumption of growth in December (Figure 4).

(iv) The maximum of growth was observed in August for the crop on board and in May for the cultivation in uncropped soil, most probably because the boards retain water longer than the non-worked soil; and growth continues at the beginning of the dry season (July), so for uncropped soil, the growth stops with the end of the rainy season (May). It is also noted that this maximun is not found until December (beginning of the following rainy season) after a severe water stress during the dry season (June-September). The best time for the first harvest after planting is **June-August** and if this harvest is done, as is desirable, for 6-7 month old plant, it suggests planting 6 and 7 months earlier, that is, in **November-December.**

(v) The number of offshoots per plant (tall) varies from 4 to 22 on board from March to December and from 7 to 15, on uncropped soil, from May to December (Figure 5). The board cultivation is always advantageous. The number of leaves per offshoot ranged from 23 to 115 on board from March to December and from 30 to 95 on uncropped soil from May to December.

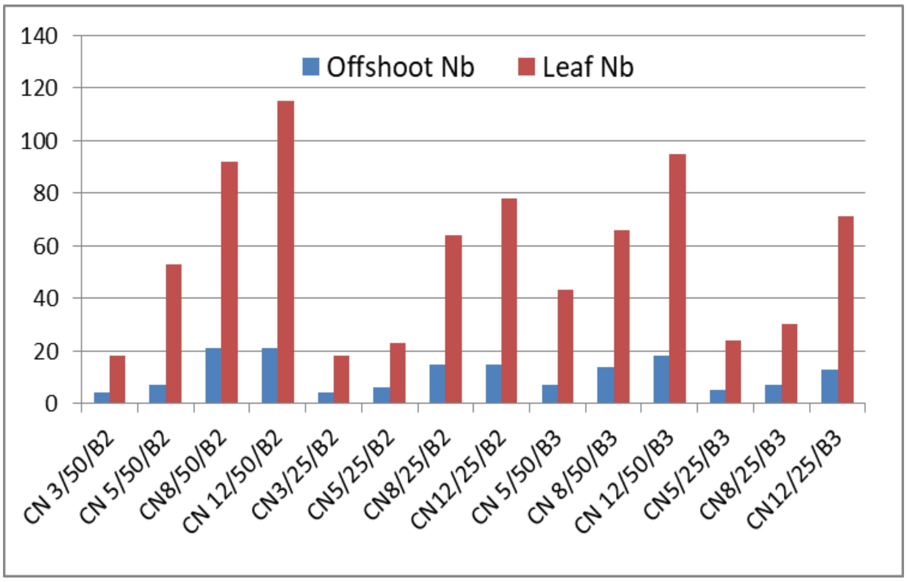

Figure 5. Leaf and offshoot production by *C. nardus* in the experimental field at Nkama

The correlation between the number of offshoots per plant and the number of leaves per offshoot is given in the figure 6 :

(number of offshoots per foot) = 5.25 (number of leaves per shoot) - 1.189

with $R^2 = 0.9375$

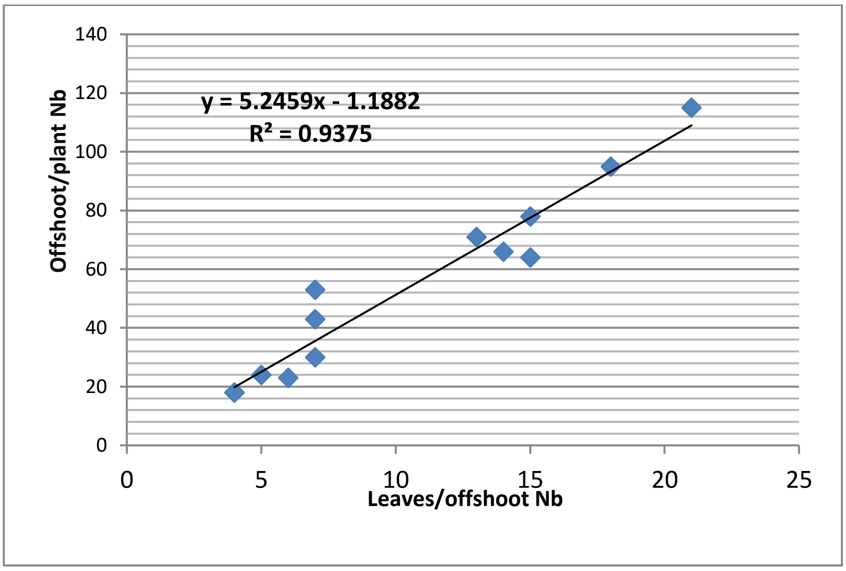

Figure 6. Correlation <offshoot- leaves> of *C. nardus* in the experimental field of Nkama

3.2. Effects of factors: soil working, planting spacing and harvest month on aerial biomass production

Tables 4 and 5 give respectively the experiment matrix and the experiment design with the responses of the complete factorial design 2^3 for the production of foliar biomass by *C. nardus.*

Tableau 4. Experiment matrix of complet factorial design 2^3 for foliar biomass production by *C. nardus.*

Experiment run	Harvest month	Ecartement (cm)	Soil work
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
Level -1	5	25	cropped B2
Level +1	12	50	uncropped B3

Table 5. Experiment design and responses of complet factorial design 2^3 for foliar biomass production by *C. nardus.*

Experiment run	Harverst month	Planting spacing (cm)	Soil work	Biomass production (t/ha)	Number offshoots per foot	Number Leaves per offshoot
1	5	25	cropped B2	2,9	8	10
2	12	25	cropped B2	7,0	15	78
3	5	50	cropped B2	1,6	3	7
4	12	50	cropped B2	5,2	21	115
5	5	25	uncropped B3	1,2	5	6
6	12	25	uncropped B3	6,6	14	6
7	5	50	uncropped B3	0,58	4	7
8	12	50	uncropped B3	3,4	20	6

Let's look in detail at the response < folial biomass production (FBP)>.

The figure 6 validates the model: RMSE = 0.7354; $\mathbf{R^2 = 0.99}$; p-value = 0.2097.

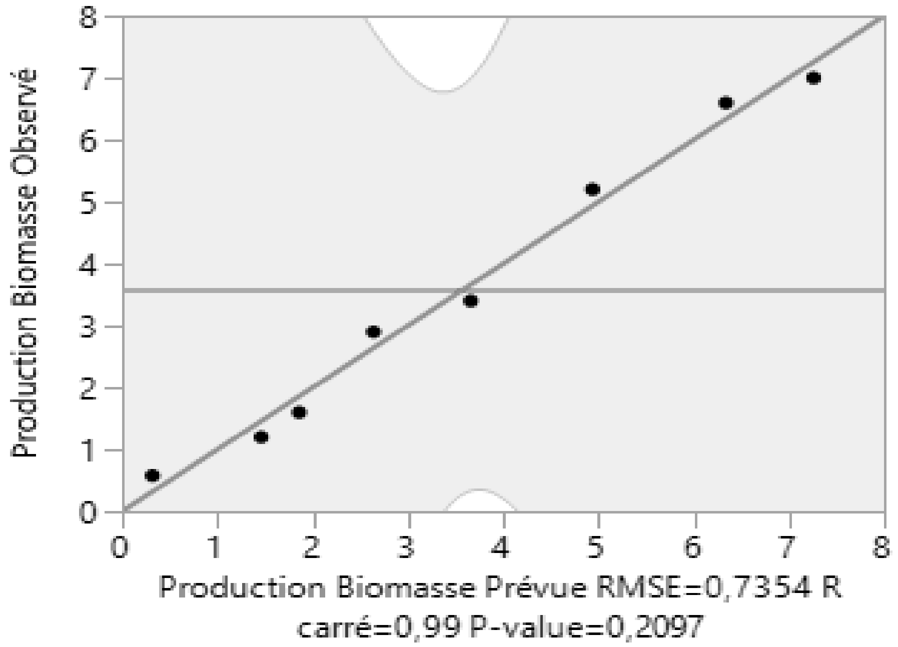

Figure 6. Correlation between predicted production and observed production for *C. nardus* biomass production at Nkama

Table 6 reports estimated values of the coefficients of factor and interaction effects in foliar biomass production from *C. nardus* thanks to JMP SAS software. The equation of the yield response is therefore:

$$y = 3.56 + 1.99x_1 - 0.87x_2 + 0,62x_3 - 0.39x_1x_2 + 0.07x_1x_3 - 0.09x_2x_3$$

The F values (Table 7) lead to the following equation:

$$\mathbf{y = 3.56 + 1.99x_1 - 0.86x_2 - 0.61x_3 - 0.39x_1x_2}$$

Table 6. Estimated values of the coefficients of factor and interaction effects in foliar biomass production by *C. nardus*

Term	Estimation	t ratio	Prob. > |t|
Constant	3,56	13,69	0,0464*
Harvest month (x_1)	1,99	7,65	0,0827
Planting spacing (x_2)	-0,865	-3,33	0,1859
Soil work (x_3)	0,615	2,37	0,2546
Harvest month * Planting spacing (x_1 x_2)	-0,385	-1,48	0,3781
Harvest month * Soil work (x_1x_3)	-0,065	-0,25	0,8440
Planting spacing * Soil work (x_2x_3)	0,09	0,35	0,7879

Tableau 7. F tests of factor and interaction effects for for *C. nardus* biomass production at Nkama

Source	F ratio	**Prob. > F**
Harvest month (x_1)	**58,5814**	**0,0827**
Planting spacing (x_2)	**11,0684**	**0,1859**
Soil work (x_3)	**5,5950**	**0,2546**
Harvest month * Planting spacing (x_1 x_2)	**2,1927**	**0,3781**
Harvest month * Soil work (x_1x_3)	0,0625	0,8440
Planting spacing * Soil work (x_2x_3)	0,1198	0,7879

The model leads to a prediction of average leaf biomass production of 3.56t / ha with <harvest month factor> as the most positive influential on leaf biomass production (1.99); the factor <planting spacing> has a negative effect (- 0.87). The effect x_3 and the interaction x_1 x_2 produce negligible positive (0.615) and negligible negative (- 0.39) effects. Models validated by expriments of all the responses were gathered in table 8 with R^2 and F corresponding values.

Table 8: Models validated by the experiments

Models	Variables	R^2	F value
FBP = $3.56 + 1.99x_1 - 0.86x_2 - 0.61x_3 - 0.39x_1x_2$	Harvest month (x_1) Planting spacing (x_2) Soil work (x_3)	0.99	$F(x_1) = 59$ $F(x_2) = 11$ $F(x_3) = 6$
NOP = $11.3 + 6.3x_1 + 2.3x_1x_2$	Harvest month (x_1) Planting spacing (x_2)	0.99	$F(x_1) = 156$ $F(x_2) = 20$
NLO = $29{,}375 + 21{,}875x_1 + 23{,}125\ x_3 + 22{,}125\ x_2x_3$	Harvest month (x_1) Soil work (x_3)	0.98	$F(x_1) = 18$ $F(x_3) = 20$ $F(x_1x_3) = 19$

FBP : folial biomass production; NOP : Number of offshoots per plant ; NLO: Number of Leaves per offshoot.

Figure 7 representing the factor effects shows :

(i) the significant effects of the harvest month on responses ,

(ii) no effects of the planting spacing

and (iii) the effects on soil work which decreases in absolute value in the direction: leaves / offshoot> biomass > offshoot / plant. So, it appears that on a 12-month follow-up crop, the best results are expected at 8.5 months for a spacing of 37.5 cm, with an average production of 4.2 t / ha of leaf biomass, 12 offshoot/ plant and 53 leaves /offshoot.

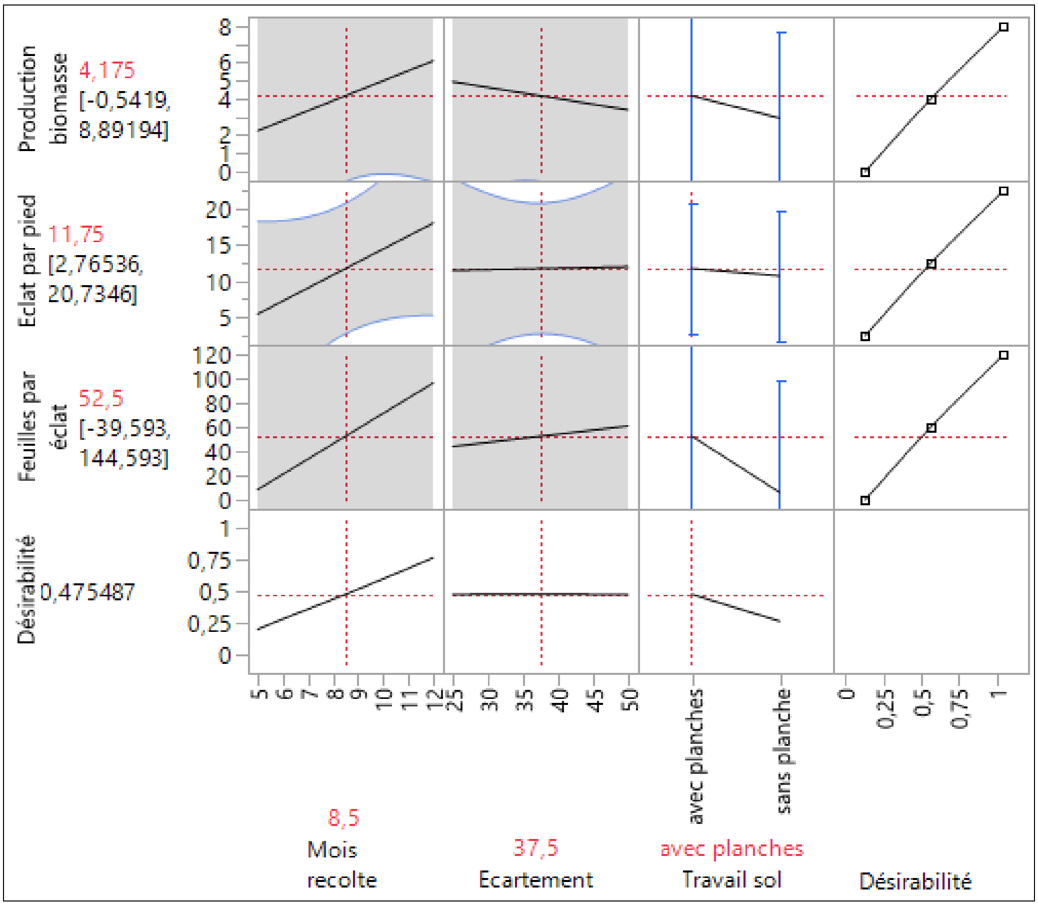

Figure 7. Graphical representation of the factor effects on biomass production

3. Modeling the production of essential oil

The essential oil yield, noted y depends on the variables (factors) X_1, X_2, X_3 and their variation levels are reported in table 9, experiment matrix and experiment design, in tables 10 and 11.

Table 9. Variation levels of factors in the complet factorial design 2^3.

Levels	Harvest period (X_1)	Quantity (kg) of biomass (X_2)	Extraction Duration (h) (X_3)
Low (-1)	8-9 h	10	2
High (+)	15-16 h	25	6

Tableau 10. Experiment matrix

Experiment run	Harvest period (x_1)	Quantity (kg) of biomass (x_2)	Extraction Duration (h) (x_3)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
Level -1	8-9 h	10	2
Level +1	15-16 h	25	6

Tableau 11: Design of Experiment

Experiment run	Harvest period (X_1)	Quantity (kg) of biomass (X_2)	Extraction Duration (h) (X_3)	Yield (EO). y %
1	9	10	2	2.0
2	15	10	2	1.95
3	9	25	2	1.04
4	15	25	2	1.14
5	9	10	6	2.6
6	15	10	6	2.85
7	9	25	6	2.0
8	15	25	6	2.16

We have a three-factor function that is written: $y = f(X_1, X_2, X_3)$. It became, after using factor and interaction coefficients reported in table 12

$$y = 1.97 + 0.0575X_1 - 0.3825X_2 + 0.435X_3 + 0.0075X_1X_2 + 0.045X_1X_3 + 0.06X_2X_3$$

Tableau 12. Estimated values of the coefficients of factor and interaction effects in essential oil production by *C. nardus* acclimatized in Nkama

Terms	Estimated values	t ratio	Prob. > |t|
Constant	1,9675	**65,58**	**0,0097***
Harvest period (x_1)	0,0575	1,92	0,3061
Quantity of biomass(kg) (x_1)	-0,3825	**-12,75**	**0,0498***
Distillation duration (x_3)	0,435	**14,50**	**0,0438***
Harvest period * Quantity(x_1 x_2)	0,0075	0,25	0,8440
Harvest period * Dist. duration (x_1x_3)	0,045	1,50	0,3743
Quantity * Dist. duration (x_2x_3)	0,06	2,00	0,2952

The results in Figure 8 validate the model with RMSE = 0.0849; $\mathbf{R^2 = 1.00}$; p-value = 0.0955.

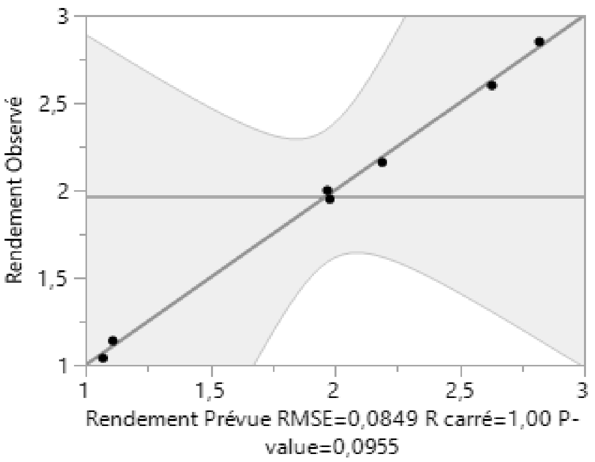

Figure 8. Correlation between predicted extraction yield and observed extraction yield for *C. nardus* essential oil extraction at Nkama

The tests t (table 12 and F (table 13) indicate that only the constant, the <distilled quantity> and <distillation time> factors are statistically significant: the equation of the model is reduced to:

$$\mathbf{y = 1.97 - 0.38x_2 + 0.44x_3}$$

Tableau 13. F tests of factor and interaction effects for *C. nardus* Essential oil production at Nkama

Source	Rapport F	Prob. > F
Harvest period (x_1)	3,6736	0,3061
Quantity of biomass(kg) (x_1)	**162,5625**	**0,0498***
Distillation duration (x_3)	**210,2500**	**0,0438***
Harvest period * Quantity(x_1 x_2)	0,0625	0,8440
Harvest period * Dist. duration (x_1x_3)	2,2500	0,3743
Quantity * Dist. duration (x_2x_3)	4,0000	0,2952

Figure 9 giving the optimal conditions supply profile shows that in the experimental field considered: (i) the extraction yield is insensitive to the item of harvest of the plant material (ii) the yield decreases with the high packing of the plant material in the distiller, (iii) the yield increases with the duration of the distillation. In conclusion, under the operating conditions selected, an average yield of 2 % is excepted from 2.5 kg of plant material harvested at 12.00, dried for a week and distilled for 4 h.

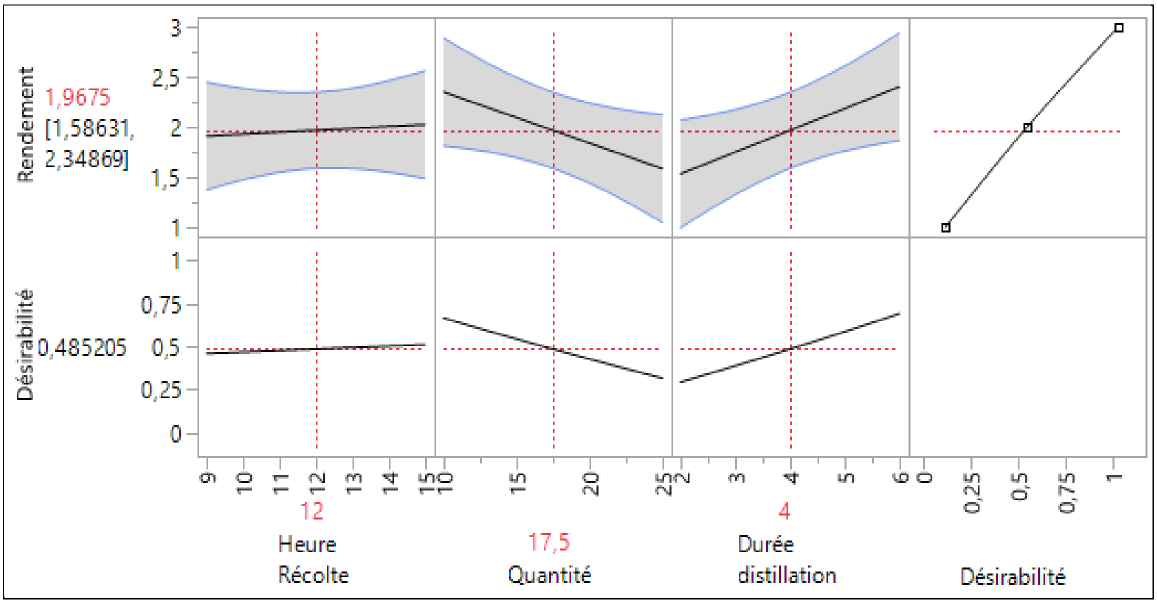

Figure 9. Graphical representation of the factor effects on hydrodistillation of *C. nardus*

4. Characterization of the essential oil of C. nardus produced in Nkama

The species *Cymbopogon nardus* acclimatized on the “plateau des Cataractes” came from Benin under the name *C. nardus* "java type"; it has adapted perfectly to this new ecosystem [1][3]. However, we proceeded, for verification, to the analysis of the chemotype of the offshoots used for setting up of experimental field of Nkama. Table 14 compares the composition of 4 oil samples extracted from this offshoot. Forty-nine (49) constituents representing 99.42 % of the total oil were identified: Three major compounds, with individual contents varying from 10 to 46% and 8 minor constituents with individual contents varying between 1 % and 4 % of the total oil. Table 15, representing the multi-annual variation of the average chemical composition (M ± SD %) of major essential oil constituents of the of *Cymbopogon nardus* acclimatized on the “plateau des Cataractes”, confirm the stability of the chemotype of essential oils produced. Figure 10 (oil in raw offshoots) and 11 (oil produced in the experimental field) represent the standard chromatograms of the oils extracted from *C. nardus* acclimatized on the “plateau des Cataractes”, in Congo-Brazzaville. One observes the similar chemical profile: Citronella chemotype characterized by the same firstmajors constituents (citronellal, geraniol, citronellol) accounting for more than 75 % of the total composition of the essential oil.

Tableau 14 : Chemical composition of *C. nardus* essential oils acclimatized in «plateau des Cataractes» used as raw offshots in setting up of experimental field.

	RI_C	IR_{Lit}	Constituents	CN 25/15 LP	CN 17/15 NKv	CN 18/15 LP	CN 22/15 Lc	Mean (standard deviation)	Codes of main constituents
1	**1028**	**1024**	**Limonene**	**3,34**	**2,90**	**2,62**	**3,07**	3,0 (0,3)	**1**
2	1100	1004	Mentha-1(7),8-diène	0,58	0,51	0,50	0,53	-	
3	**1150**	**1145**	**Isopulegol**	**1,08**	**1,22**	**0,89**	**0,89**	-	**2**
4	**1156**	**1154**	**Citronellal**	**43,54**	**45,45**	**43,13**	**43,70**	44,0 (0,9)	**3**
5	1160		*iso* Isopulegol	0,85	0,92	0,46	0,59	-	
6	1171		*néo-iso* Isopulegol	0,10	0,10	0,12	-	-	
7	**1229**	**1228**	**Citronellol**	**10,46**	**11,99**	**10,78**	**10,55**	10,9 (0,6)	**4**
8	1240	1235	Neral	0,53	0,43	0,48	0,44		
9	**1254**	**1255**	**Géraniol**	**22,02**	**21,48**	**24,48**	**23,00**	22,7 (1,1)	**5**
10	1261	-	NI	-	0,31	0,32	0,31	-	
11	1270		Géranial	0,69	0,49	0,60	0,60	-	
12	1343	-	NI	0,20	0,24	0,16	0,20	-	
13	**1349**	**1350**	**Citronellyle acétate**	**1,23**	**1,22**	**1,45**	**1,76**	1,4 (0,2)	6
14	1353	1356	Eugénol	0,67	0,67	0,71	0,90	-	
15	**1378**		**Geranyle acétate**	**1,31**	**1,06**	**1,96**	**2,42**	1,7 (0,5)	**7**
16	1381	-	NI	0,12	0,12	0,12	0,12	-	
17	1383	1387	β-Bourbonene	0,10	0,09	0,10	0,12	-	
18	**1389**	**1389**	**β-Elemene**	**1,70**	**1,87**	**1,61**	**1,51**	1,7(0,1)	**8**
19	1456	1452	α-Humulene	0,08	0,08	0,08	0,07	-	
20	1475	1478	γ-Humulene	0,15	0,16	0,14	0,14	-	
21	**1481**	**1484**	**Germacrene-D**	**1,41**	**1,36**	**1,29**	**1,48**	1,4(0,1)	**9**
22	1498		β-Cadinene	0,20	0,46	0,43	0,43	-	
23	1508	1508	Germacrene-A	0,31	0,30	0,28	0,29	-	
24	1513	1513	γ-Cadinene	0,36	0,36	0,34	0,33	-	
25	**1518**	**1522**	**δ-Cadinene**	**1,45**	**1,66**	**1,61**	**1,56**	1,6 (0,1)	**10**
26	1537	1537	α-Cadinene	0,08	0,09	0,08	0,08	-	
27	**1550**	**1549**	**Elemol**	**2,31**	**2,60**	**2,83**	**2,70**	2,6 (0,2)	**11**
28	1578		γ-Eudesmol	0,43	0,27	0,65	0,54	-	
29	1634	1638	epi α-cadinol	0,20	0,25	0,26	0,23	-	
30	1646	1644	α-Muurolol	0,15	0,44	0,21	0,35	-	
31	1658	1652	α-Eudesmol	0,68	0,80	0,89	0,88	-	
32	1676	-	NI	0,11	0,10	0,11	0,13	-	
			Total	**96,44**	**100,0**	**99,69**	**99,92**		
Monoterpene hydrocarbons				3,92	3,41	3,41	3,6		
Oxygenated monoterpenes				82,68	85,58	85,58	85,36		
Sesquiterpene hydrocarbons				5,96	6,55	6,55	6,13		
Oxygenated sesquiterpenes				3,88	4,48	4,48	4,83		

Tableau 15. Pluri-annual variation of the average chemical composition (M ± SD%) of major essential oil constituents of the of *Cymbopogon nardus* acclimatized on the "plateau des Cataractes" [1] [3].

Constituents	Year (sample size)					
	2003 (n= 10)	2012 (n= 10)	2013 (n= 7)	2015 (n=11)	2016 (n= 14)	2017 (n= 12)
Limonene	2,64 (1,2)	2,39 (1,0)	2,18 (0,17)	3,02 (0,3)	2,99 (0,2)	3.56 (0.1)
Citronellal	39,04 (9,1)	44,14 (2,6)	33,68 (4,78)	44,87 (2,1)	43,91 (3,3)	48.70 (1.8)
Citronellol	10,56 (3,3)	12,16 (0,8)	8,08 (1,01)	11,18 (0,5)	10,24 (0,8)	10.93 (0.8)
Géraniol	27,28 (5,1)	23,39 (1,8)	20,65 (1,71)	22,99 (1,5)	20,58 (5,8)	20.96 (0.7)
Citronellyle acétate	1,73 (1,0)	0,76 (0,4)	2,37 (0,52)	1,25 (0,4)	1,12 (0,3)	1.02 (0.1)
Géranyle ecétate	3,49 (2,8)	0,88 (0,7)	3,20 (1,48)	1,53 (0,4)	1,52 (0,6)	1.07 (0.1)
Beta elemene	0.32 (0,3)	1,18 (0,3)	2,82 (0,35)	1,66 (0,2)	1,45 (0,2)	2.06 (0.2)
Germacrene D	0,86 (0,9)	0.61 (0,3)	2,70 (0,37)	1,39 (0,1)	1,18 (0,2)	1.25 (0.1)
Elemol	2,81(1,4)	3,56 (0,8)	7,97 (2,72)	1,39 (0,1)	2,28 (0,4)	2.83 (0.4)
Germacrene D-4-ol	0,99 (0,9)	0,62 (0,2)	3,17 (0,68)	2,59 (0,3)	0,71 (0,1)	0.00 (0.0)

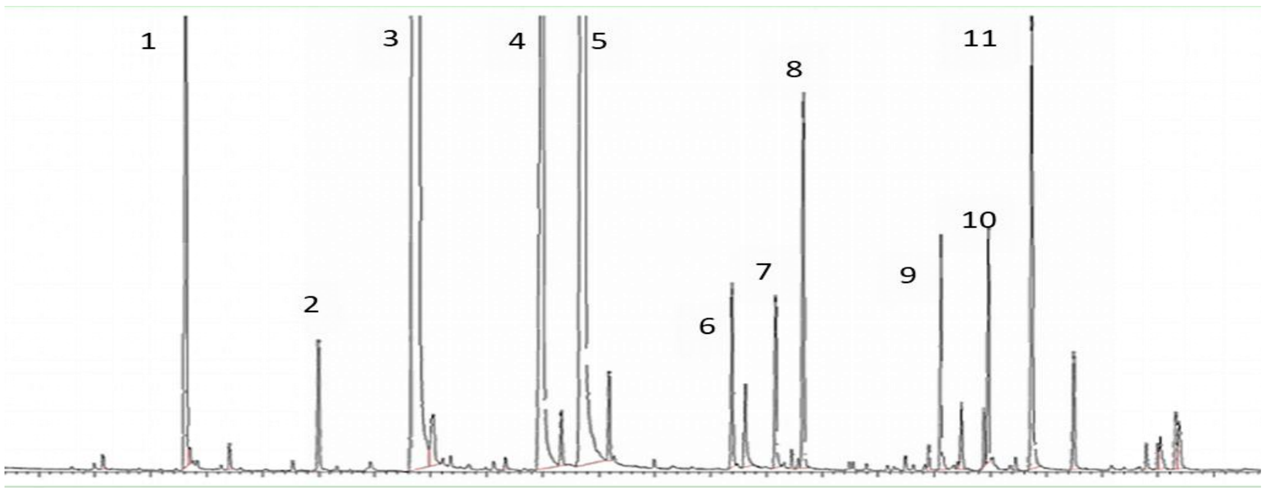

Figure 10. Standard chromatogram of oil extracted from raw material offshoots (2017) Codes of constituents: see table 17

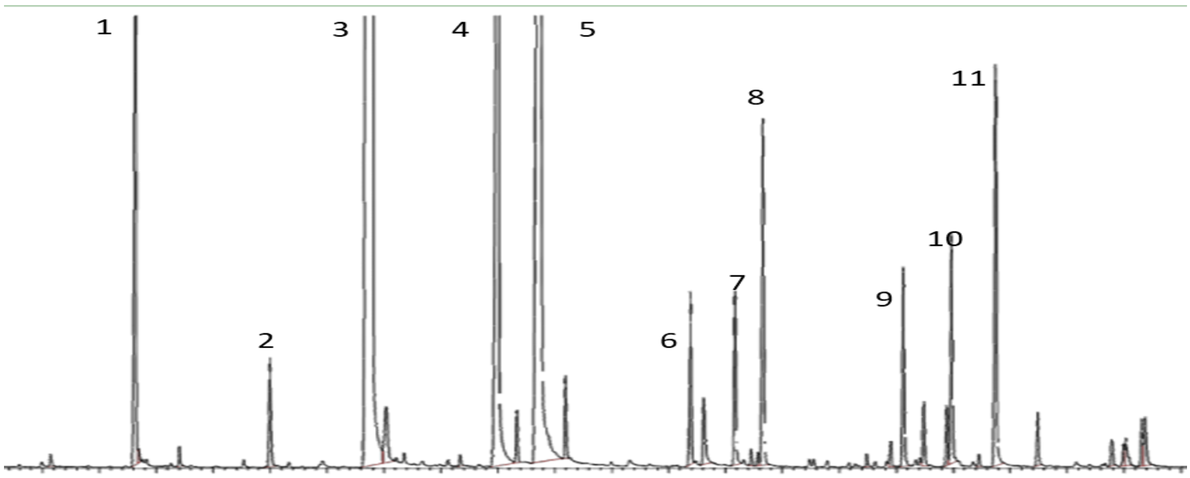

Figure 11. Standard chromatogram of oil extracted from Nkama field offshoots (2018)Codes of constituents : see table 17

Conclusion

After identification of fundamental problems related to the introduction and acclimatization of *Cymbopogon nardus* on the "plateau des Cataractes", in Congo-Brazzaville for more than a decade, this work provide practical informations on the biomass and essential oil production, on a pilot scale, particularly to producers of essential oils of citronella grass. The modeling of the phenomen thanks to a complete factorial design 2^3 made possible to establish after a follow-up of the experimental field that: (i) the best results are expected at 8.5 months for a planting spacing of 37.5 cm with an average production of 4.2 t/ha of leaf biomass, 12 offshoots/plant and 53 leaves/offshoot; (ii) the production of the essential oil is insensitive to the time of harvest in the day, but significantly sensitive to the packing of the plant material in the distiller and to the duration of the distillation; in the experimental conditions selected for this study, the best results are expected, with an average yield of 2 % of essential oil, from 12.5 kg of plant material harvested at 12.00 o' clock, dried for a week and distilled for 4 h. The essential oils extracted from offshoots of starting plant material for setting up experimental field and the those harvested on the experimental field, lead to the same citronellal chemotype, characterized by the same 3 first major constituents (citronellal (44.0 ± 0.9) geraniol (22.7 ± 1 , 1) citronellol (10.9 ± 0.6)) accounting for more than 75% of the total composition of the essential oil.

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