Variation in Yield and Composition of Essential Oil in *Pelargonium sp* During the Vegetative Stage

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***Abstract*---**The purpose of this work is to investigate the variation in yield and essential oil composition during the different phases of growth in an aromatic plant widely used in Morocco, *Pelargonium sp*. from the first week of January, which is at the dormant phase until the final week of June which is the end of blooming.

The results show a large variation in the yield of the essential oil with a maximum recorded at the stage of full blooming and end of blooming (about 1.2%). this change was accompanied by a qualitative change along of this period, with a balance of appearance and disappearance of some compounds and some change in the contents of main compounds (menthol, Isogeraniol, menthene and Eremophilene) also of the other compounds (α-pinene, hull, linalyl acetate and α-cubebene).

***Keywords*---**essential Oil, Isogeraniol, Menthol, *Pelargonium sp*, vegetative stage

I. Background

MOROCCO has a very important botanical diversity in the Mediterranean region, allowing it to produce essential oils of various plant species [1]. Among these species, the genus Pelargonium who includes about 260 species grown for their essential oils.

In India, some scientist showed that *Pelargonium* leaf extracts have repellent properties against slugs and essential oil has nematicidal activity against Meloidogyne incognita [2]. The main chemical components of pelargonuim oil are: geraniol, citronellol, isométhone, geranyl formate, citronellyl formate, linalool, Guaia-6,9-diene and cis-rose oxide [1].

The oil content of *Pelargonium* changes during the development of the plant. In the Middle East, [1] have shown that at the beginning of blooming HE content is 1% per 100 g of dry matter, gradually increasing to 1.3% in the full bloom, then decreases to 0, 6% at the end of blooming. In full bloom, the oil content is highest in flowers (3.3%), followed by leaves (1.8%). The rods contain only traces.

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The composition of these HE is strongly influenced by culture [3] and leaf age [4],Also in other aromatic crops, photoperiod, intensity of light, temperature [5] and the harvest season [6] have an important influence on the composition of terpenoids. In this contest we tried in this study to evaluate the variation in yield and essential oil composition in *Pelargonium* during the growth period extends from January to the last week of the June, which corresponds to the vegetative stages ranging from dormant until the end of blooming through the growth of the plant.

II. Material and Methods

A. Plants Material

All the tests are realized on the plants of P*elargonium sp* growing in Faculty of Science and Technology garden, for each first week of the month we measured the water contents and we dryad plants for the extraction of essential oils.

B. Relative water contents (RWC)

The relative water contents (RWC) is measured on the 7th or 8th leaf fully developed using the following formula according to Bandurska (1991):

RWC **%** = 100 × [(FW-DW)/ (WT-DW)]

With FW: the weight of fresh leaf material,

WT: the weight of fresh material from the turgid leaf was submerged in distilled water for 4 hours,

DW: dry weight of the sheet material placed in an oven at 70 ° C for 24 hours

C. Extraction of essential oils

100 g of dried aerial parts of *Pelargonuim sp* were submitted to hydrodistillation with a Clevenger-type apparatus (Clevenger 1928) and extracted with 2L of water for 180 min (until no more essential oil was obtained). The essential oil was collected, dried under anhydrous sodium sulphate and stored at 4°C until analysed. The essential oil yield is given by the following formula

YEO (ml/100 g Dm) = (V/Dm × 100) ± (ΔV/ Dm × 100)

Y.E.O: essential oil yield of dry matter

V: volume of essential oils collected (ml)

ΔV: reading error

Dm: dry plant mass (g)

D. Statistical analysis

All statistical analyses were performed with IBM.SPSS statistics, Version 19. The results of essential oils extraction and water contents were repeated three times. For the correlation we are used Pearson correlation test with level of signification at 0.01

III. Results

A. Water contents and essential oils

The yield of essential oils is changing in a major way during the growth period since found that between January and early February, a period that corresponds to dormancy phase, HE levels decrease from 0.8% to 0.4% and then increased slightly during the growth phase between March and April (0.6%). However, the yield increases very importantly since mid-April until the end of June and reaches a maximum value (1.2%) (Figure 1). This period corresponds to the blooming phase of the plant.

(Fig. 1) The water content results in the leaves show a very large variation in the different stages of plant growth: there was an increase during the dormancy phase and a steady decline from the month of February to minimum values in June, which corresponds to the end of blooming. Furthermore, there is a correlation between the water content in leaves and yield of HE (Table 1): increasing water contents rise to any increase yields HE and vice versa. This change more importantly between January and February when there is a minimum yield of HE (0.4%) and a maximum water content (81.5%), as well as at the period between May and June (1.2%) when oil content (72%) of water content (Figure 1).

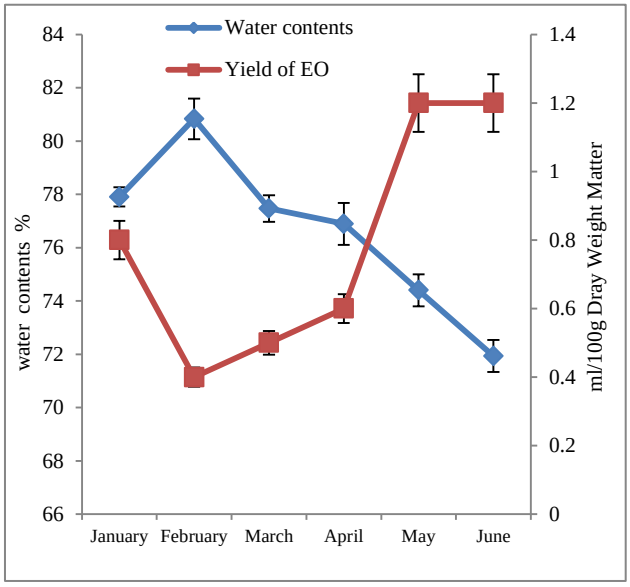

Fig. 1 Variation in water content and the yield of essential oils during the vegetative stages of *Pelargonuim sp*

TABLE I
CORRELATION BETWEEN WATER CONTENT AND ESSENTIAL OIL CONTENT IN PELARGONUIM SP (WC: WATER CONTENTS, EO: ESSENTIAL OILS)

		WC	EO
WC	Pearson correlation	1	-,840**
	Sig. (bilateral)		,000
	N	18	18
EO	Pearson correlation	-,840**	1
	Sig. (bilateral)	,000	
	N	18	18

**. The correlation is significant at the 0.01 level (bilateral).

B. Changes in the chemical composition of essential oils during different growth phases of Pelargonuim sp

TABLE II
VARIATION OF THE CHEMICAL COMPOSITION OF ESSENTIAL OILS DURING THE DIFFERENT PHASES OF GROWTH PELARGONUIM SP. DF: DORMANCY PHASE, BB: BEGINNING OF BLOOMING, FB: FULL OF BLOOMING, EB: END OF BLOOMING

RT	Compounds	Peack Area %			
		DF	BB	FB	EB
12.45	Trans-Ocimene	0.11	-	-	-
12.58	cis-Sabinene	0.20	0.16	-	0.17
12.78	1,8-Cineole	-	0.14	0.25	-
15.23	3-Carene	3.88	3.31	2.60	1.52
15.54	a-Rose oxide	0.30	0.33	0.48	1.29
15.94	á-THUJONE	0.30	0.32	0.25	0.24
16.13	b- Rose oxide	0.11	0.12	0.20	0.59
17.07	Camphor	0.51	0.44	0.49	0.43
17.17	Citronellal	-	-	0.25	0.29
17.38	p-Menthone	0.10	-	0.13	0.27
17.73	**Isoborneol**	**5.19**	**4.02**	**6.43**	**6.96**
18.22	Limonen	0.14	-	-	-
18.53	Isomenthol	-	0.14	0.13	0.15
18.65	Cryptone	0.18	-	-	-
18.81	delta.3-Carene	0.27	0.23	0.18	0.17
18.94	trans-anethole	0.12	0.15	-	-
20.03	**Menthol**	**15.80**	**14.06**	**23.43**	**20.57**
20.40	Z-Citral	0.67	0.41	0.88	0.52
20.72	cis-limonene oxide	-	-	1.44	-
20.89	**Isogeraniol**	**15.47**	**15.05**	**7.17**	**9.14**
21.48	**Menthene**	**7.87**	**6.70**	**10.4**	**9.97**
22.34	**á-Pinene**	**5.72**	**5.64**	**6.34**	**4.28**
23.74	à-Copaene	-	-	0.12	0.13
23.94	iso-menthyl acetate	0.12	0.21	0.31	0.44
24.18	b-phenylethyl acetate	0.12	0.12	0.11	0.14
24.73	à-Cubebene	0.57	0.71	0.46	0.39
24.92	á-Bourbonene	-	2.71	1.79	1.72
25.00	b-Bourbonene	1.95	-	0.18	0.20
26.18	trans-Caryophyllene	0.97	1.05	1.06	0.98
26.52	germacrene-d	0.23	0.23	0.13	0.13
26.85	aromadendrene	0.44	0.61	0.67	0.82
26.96	Naphthalene	0.16	0.17	-	-

27.12 Calarene	0.42	0.41	0.45	0.37
27.35 à-Caryophyllene	0.42	0.35	0.36	0.32
27.49 à-Humulene	0.57	0.67	0.45	0.40
27.80 delta.3-carene	1.24	1.37	1.21	1.07
28.18 à-Cubebene	3.46	4.25	3.49	2.05
28.47 Isolongifolene	1.78	2.05	1.61	1.49
28.62 Seychellene	0.39	0.76	0.42	0.42
28.94 t-bisabolene	0.54	0.53	0.50	0.49
29.16 Naphthalene	0.20	0.26	0.18	0.20
29.28 ë-Cadinene	1.06	1.16	0.93	0.98
29.45 Aromadendrene	1.05	1.48	1.02	1.53
29.73 Calarene	0.19	0.16	0.19	0.12
30.07 à-calacorene	-	-	-	0.14
30.28 trans-longipinocarveol	-	0.25	0.34	0.60
30.41 Geraniol formate	1.56	2.06	1.55	1.65
30.94 Pentalene	0.17	0.30	0.18	0.28
31.20 dehydroaromadendrene	1.07	0.55	-	0.51
31.32 octahydro-naphthalen	0.51	0.39	0.63	1.01
31.48 phenethyl tiglate	1.67	1.64	1.71	1.97
31.70 Azulene	0.82	0.52	0.40	0.23
31.99 Junipene	0.20	1.23	0.24	0.37
32.16 Ledene oxide	0.17	0.28	0.10	0.18
32.28 Cubenol	0.30	0.35	0.35	0.46
32.55 Eremophilene	**8.34**	**9.02**	**7.39**	**8.19**
32.88 Valencene	0.63	0.82	0.70	0.83
33.05 Copaene	0.25	0.38	0.28	0.34
33.19 á-Guaiene	0.88	1.06	0.92	1.20
33.47 Elemol	1.86	2.30	1.98	2.32
33.64 ç-Gurjunenepoxide	0.27	0.28	0.41	1.20
33.96 Ethyl linoleate	0.17	0.21	0.14	0.31
34.50 linalyl acetate	3.38	3.90	2.88	2.97
34.67 ç-himachalene	-	-	-	0.13
34.88 Camphene	0.39	0.44	0.27	0.38

The essential oils of *Pelargonium* are characterized by a high heterogeneity in the composition with 65 molecules), 4 majority (Menthol, Isogeraniol, menthene and Eremophilene). This composition changes importantly, both qualitatively and quantitatively, in the vegetative stages.

Note that a balance of appearance and disappearance of certain molecules was noticed during the growth of these plants, and a remarkable change in the levels of pre-existing molecules.

Qualitatively: we see that the period of June which is the end of blooming has the most heterogeneous composition with more than 58 molecules, also the composition remains standard at dormant phase (from January until early Mars) and full bloom (late April until May) (56 molecules), however 57 molecules were detected in the beginning of blooming (mid-March to the end of April).

When the plant is in a vegetative state (dormant phase and growth phase) we note the absence of certain molecules that appear with the beginning of blooming: the case of isomenthol, α -bourbonene and trans-longipinocarveol. In addition, other molecules are present only in the dormant phase and disappear with the beginning of blooming: the case of trans-ocimene, limonen and cryptone.

Quantitatively: Main and secondary compounds undergo multitudes variations in levels that reflect a major change in biosynthetic plants. The levels of major compound (menthol) hardly changed during the early stages in which correspondent dormant growth and beginning of blooming) on the other side, from full blooming, there is an important increase that can reach about 48% and 30% respectively during the stage of full blooming and late blooming. Increased levels of this molecule is inversely proportional to the decline of isogeraniol (Figure 2), since we noticed that the contents of the latter remain stable during the first two stages and decrease of about 54% and 41% respectively during the FP and FF stages.

Furthermore, the contents of menthene increase in a remarkable way from the full of blooming and remains stable until the end of blooming, while the contents eremophilene show no change. Secondary compounds have also undergone a multitudes changes, since noticed that the β-bourbonene and dehydro-aromadendrene that are present in important quantities in dormant phase, decline from the beginning of blooming while the 3-carene steadily decreasing from dormant phase until the end of blooming.

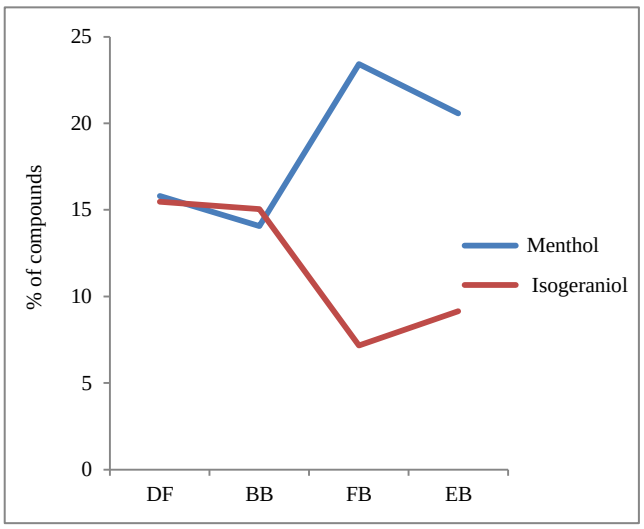

Fig. 2 Changes in levels of menthol and isogeraniol during the vegetative stages of *Pelargonuim sp*

IV. Discussion

The variation of water content in leaves of *Pelargonium sp* plants is directly related to climatic conditions (precipitation and temperature). In Morocco, these two factors undergo significant fluctuations since the month of January to end of June. Between January and March, substantial rainfall and very low temperatures were recorded, which explains the increase in water content in the plant. Furthermore, the months of May and June are characterized by high temperatures and low rainfall which causes a plant dehydration.

The results showed that the yield of essential oils varies during the growing cycle of *Pelargonium*: It decreases during the dormancy phase and increases during the growth phase and beginning of blooming to stabilize from full of blooming until the end of blooming. This result confirms those found by other authors who have shown that yields of HE increase along the vegetative stages. [1] Others have

shown that HE yields are vary depending on weather conditions [7] and the number of secretory glands [8]

In general, in the aromatic cultures, essential oil is biosynthesized as a secondary metabolite from the products of photosynthesis [8].Low photosynthetic activity negatively affects the synthesis of essential oils and their accumulations. The low levels of essential oil during the first two stages, seem to be due to: (a) limited availability of photosynthesis since this period corresponds to a dormant plant (low light, low temperature and therefore low photosynthesis) [9]; (b) catabolism of essential oil [8]in response to seasonal stress (temperature drop).

According to some authors, the transition from the vegetative stage of a plant to another is performed following a physiological change triggered by the increase of the light intensity [13]: in the last two stages (PF and FF) , the plants are in adequate conditions for the growth with an extended illumination period and high temperatures, which leads to an increased number of glands leaves [9] ,and pushes the plants synthesize quantities more important flavors that will serve to the physiological responses to climatic conditions [7] and the attraction of insects for pollination.

These results also show that the chemical composition of terpenoids is strongly influenced by climate change and vegetative stages: In *Pelargonuim sp*, winter favors the accumulation of large amounts of geraniol and its esters, while the summer season favors accumulation Menthol and its esters. The humidity and rising temperatures coupled with high light intensities during the summer probably lead to the conversion of part of the isogéraniol to menthol [10]. This change in chemical composition due to weather conditions has also been reported by [9] and [3] have shown that *Pelargonium* plants subjected to water stress can lead to an essential oil rich of citronellol. Moreover, the decline of secondary compounds contents can be explained either by catabolism that occurs after the changes undergo by the plant following the multitudes growth period, either as a result of adverse conditions that may occur during growth [8].

V. Conclusion

Further studies are needed to shed light on the relationship between the metabolism of plants during growth, vegetative stages and climate change related to each period, however, in this study we found a correlation between the yield of essential oils and water contents, this change is reflected even at the level of the composition, with the appearance of new molecules and the disappearance of others.

Catabolism reactions may change the contents of a few molecules in others, this is the case of menthol, menthene and Isogeraniol, moreover there are molecules which retain stable levels along this period of growth and whatever the climatic conditions (Eremophilene).

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