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Characterization of Essential Oil and Effects on Growth of *Verbena gratissima* Plants Treated with Homeopathic Phosphorus

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Plant models offer a method to examine the efficacy of homeopathic solutions. Homeopathic *Phosphorus* (P) dynamizations were evaluated on the linear growth and dry biomass of *Verbena gratissima*, a plant native to Brazil. The yields and chemical characterization of the essential oil are also given. Plants exhibited phenotypic plasticity after the homeopathic *Phosphorus* treatments. The dynamization 9CH, in particular, interfered with plant growth, height, diameter of stems and total dry mass. 9CH treatment showed the highest yield of essential oil. The essential oil composition of *V. gratissima* varied according to the different dynamization used. Homeopathic *Phosphorus* provided the greatest amount of β-pinene, *trans*-pinocarveol, *trans*-pinocamphone and *trans*-pinocarvyl acetate in comparison with controls.

Keywords: *Aloysia gratissima*, Brazilian-lavender, ultra-high dilutions, agro-homeopathy, CG-MS, dendrogram, terpenes.

Verbena gratissima (Gillies ex Hook) Troncoso (*Aloysia gratissima*) is a perennial shrub used in folk medicine for the treatment of bronchial infections, lung diseases, and bladder disorders. It is also used as an antimicrobial agent and as flavoring for infusions and meat [1,2].

According to Brunini, chemical fertilizers and pesticides have low energy and high chemical composition. When an organism is influenced by such low energy, its own internal energy is unbalanced and can cause outbreaks of symptoms. On the contrary, homeopathic medicine is highly energetic and "beings treated by homeopathy are less vulnerable to disease" [3]. Because of the extreme dilutions used, the environmental impact is low and such treatments are well suited to the holistic approach of sustainable agriculture [4].

Experiments with plants are possible without the disadvantages of clinical trials (such as placebo effect, ethical difficulties, duration of experiments and high costs). Moreover, plant-based bioassays rely on a very cheap and almost inexhaustible source of biological material. This is a very important feature because it allows a large number of experimental repetitions to be performed, and is useful for overcoming the problem of irreproducibility so often reported in homeopathic literature [4].

The main part of the treatments uses highly diluted remedies, sometimes beyond the Avogadro-number border. Moreover, scientific studies have started to confirm the effects of high dilutions in homeopathic therapy [5].

Experimental works with the use of homeopathy in plants are rare. More studies are present in the literature about the use of homeopathic *Phosphorus* 3CH, which increased the content of coumarin in *Justicia pectoralis* Jacq plants up to 40.5% [6]. This is in agreement with the hypothesis that homeopathy interferes significantly with the metabolism of plant defense.

The element *phosphorus* (P) is essential for plant growth and development, and the Brazilian soil is poor in this element. The term P refers to the chemical itself, while *Phosphorus* refers to homeopathy, being a Greek term, where *Phospho* means light and *phorus* translates as ‘to carry’, so *Phosphorus* means "the light carrier". The homeopathic remedy *Phosphorus* covers many symptoms, because it was prescribed for hypersensitive organisms in plants with light sensitivity and as a defense against different attacks [7,8].

Homeopathic *Phosphorus* is recommended in cases of excessive sweating for heat intolerance of plant species or varieties. When demanding plants are not adequately fertilized, they do not respond to growth. If *Phosphorus* is used, the plant growth is identical to that of the fertilized ones [9].

The objective of this study was to investigate the response of *V. gratissima* to *Phosphorus* in terms of balance between growth and production of terpenes and pathogenesis. According to the observed results, *V. gratissima* is sensitive to energy homeopathic *Phosphorus*, both in growth and in production of biomass (Table 1).

The height of *V. gratissima* plants was significantly higher than the controls for dynamizations of the homeopathic *Phosphorus* 9CH, 21CH and 27CH (143.5, 139.9 and 145.7 cm, respectively) (Table 1).

Regarding the diameter of the stems, almost all the dynamizations tested (6CH, 9CH, 18CH, 21CH, 27CH, 30CH and for the control ethanol 70°GL) significantly increased the width of the stems, ranging between 10.8 mm and 11.9 mm (Table 1). Since the diameter of the stems is correlated directly with the capacity for transporting water and carbohydrates [10] and indirectly with the capacity for storing these metabolites [11], these homeopathic *Phosphorus* dynamizations positively influenced plant growth.

Growth is influenced by the quality and quantity of light, which interferes with the photosynthetic process, causing variations in plant biomass. In this study, even in a greenhouse, the use of homeopathic *Phosphorus* in dynamizations 6CH, 9CH and 27CH probably interfered with the photosynthetic process and considerably increased the total biomass of plants (110.1, 116.0, 111.6 g, respectively) in comparison with the two controls that had not been subjected to the stimulus of the energy normally used for homeopathic solutions (Table 1).

Brazilian-lavender is influenced by dynamizations in different vegetative parts. About the root biomass, a large number of dynamizations studied influenced the weight, such as 5CH, 6CH, 9CH, 18CH, 27CH, 30CH and control with ethanol 70°GL, where the values ranged between 30.0 g and 33.5 g (Table 1). Dynamization *Phosphorus* 9CH evidenced the highest dry weight of leaves (Table 1). This result is desirable in biological agriculture of medicinal plants, since the elaboration of dynamization 9CH is simple and easy to use for a rational cultivation of *V. gratissima*.

Only the control with water showed the highest value for the root: shoot ratio (0.59) (Table 1). This may be indicative of specialization to different environments. In general, more shaded environments gave a higher allocation to leaves. The homeopathic *Phosphorus* dynamization did not influence this parameter for *V. gratissima* plants.

The increased biomass of shoots (leaves and stems) was achieved at the expense of the root biomass, so the use of homeopathic *Phosphorus* apparently provided a physiological balance of *V. gratissima* plants, which, despite the growth under greenhouse conditions, is more evident in the dynamizations 6CH, 9CH and 27CH (77.7, 83.4, 81.0 g, respectively) (Table 1). Among the physiological aspects, the leaf weight ratio represents the ability of translocation of assimilates from shoots to the rest of the plant. So, if this ratio is higher than in the controls, more efficient translocation occurs and favors the increase in stem diameter. Regarding the leaf weight ratio, the effects of 9CH, 12CH, 15CH, 18CH and 27CH (0.59, 0.58, 0.59, 0.60, 0.56, respectively) were similar and significantly higher than that produced by other dynamizations (Table 1). For the variables of content and yield of oil, only dynamization 9CH presented higher values. This is in agreement with the results of growth and biomass, where bigger plants with a greater mass produce larger quantities of essential oil of Brazilian-lavender (Table 1).

The homeopathic solutions caused positive and negative variations in the linear growth and production of biomass of *V. gratissima* plants, when compared with controls. This behavior is typical in homeopathy and the same substances can switch from positive to negative effects, according to the stimulation, the solution used, the dynamization and the affinity to the organism, known as “the wave phenomenon”, common in Nature in the electromagnetic spectrum, in the tides, and in many other examples [12].

Regarding the evaluation of the essential oil production in *Verbena gratissima*, the yields ranged between 0.30 to 0.72%, and the major production was shown by dynamization 9CH, which demonstrated the best efficiency of essential oil production (mL/dry weight plant) (35.2) (Table 1).

The effects of homeopathy were observed in basil plants (*Ocimum basilicum* L.) in which the content of essential oil (yield %) in plants treated separately with homeopathic *Sulphur* 30CH (1.1%), *Calcarea carbonica* 30CH (1.1%), *Carbo vegetalis* 30CH (1.3%), and *Phosphorus* 30CH (0.7%) was lower than in the control treated with distilled water (1.7%) [13]. As with *O. basilicum* plants, *V. gratissima* was also not influenced by homeopathic *Phosphorus* 30CH, since this gave a low yield of essential oil in comparison with *Phosphorus* 9CH dynamization (Table 1).

Lemon grass (*Cymbopogon citratus*) plants, after the use of ISO 12C (isoterapic of lemongrass), produced the highest yield (2.1%) of essential oil from the leaves, while treatment with homeopathic *Sulphur* 200C resulted in the smallest one (1.3%). This work showed that the use of homeopathic

Table 1: Effects of different dynamizations of *Phosphorus* together with two controls (distilled water and ethanol 70° GL) on linear growth, production of biomass and essential oil yield and efficiency in *Verbena gratissima* plants.

	Height (cm)	Diameter of stems (mm)	Dry mass (g)				Root:shoot ratio	Leaf weight ratio	Yield** (%,v/w)	Efficiency*
			Shoot	Leaves	Roots	Total				
5CH	130.7 c	9.9 b	63.9 d	34.5 d	30.0 a	93.9 d	0.5 b	0.5 b	0.5 f	17.3 e
6CH	134.0 b	11.9 a	77.7 a	41.7 b	32.4 a	110.1 a	0.4 c	0.5 b	0.6 c	25.9 c
9CH	143.5 a	11.1 a	83.4 a	49.1 a	32.6 a	116.0 a	0.4 c	0.6 a	0.7 a	35.2 a
12CH	136.2 b	10.1 b	73.9 b	42.8 b	27.2 b	101.1 c	0.4 c	0.6 a	0.7 b	29.8 b
15CH	124.9 c	10.3 b	67.9 c	39.9 c	27.2 b	95.1 d	0.4 c	0.6 a	0.6 e	22.5 d
18CH	130.3 c	10.8 a	75.3 b	45.1 b	30.2 a	105.4 b	0.4 c	0.6 a	0.6 c	28.7 b
21CH	139.9 a	11.6 a	73.7 b	39.5 c	27.8 b	101.6 c	0.4 c	0.5 b	0.6 e	22.5 d
24CH	120.7 d	9.1 c	55.8 e	28.2 e	20.8 c	76.7 f	0.4 c	0.5 c	0.4 e	12.7 f
27CH	145.7 a	11.2 a	81.0 a	45.2 b	30.7 a	111.6 a	0.4 c	0.6 a	0.3 f	13.8 f
30CH	129.1 c	11.2 a	58.3 e	29.8 e	30.0 a	88.4 e	0.5 b	0.5 c	0.6 d	17.9 e
H_2O	102.2 e	8.4 c	41.7 f	19.1 f	28.0 b	69.7 f	0.6 a	0.4 d	0.6 e	11.1 g
Ethanol	130.7 c	11.4 a	71.8 b	36.0 d	33.5 a	105.3 b	0.5 b	0.5 c	0.7 b	24.7 c
CV	5.22	7.53	7.39	10.43	15.27	7.97	10.56	6.31	3.70	10.56

Means followed by same letter in vertical, not statistically different among themselves, by Scott-Knott test ($p \leq 0.05$). CV = coefficient of variation (%).
* Efficiency = mL·dry weight plant. ** Dry mass.

ISO in the lower dynamization (ISO12C) was better than the use of dynamization (ISO 200C) to produce good amounts of essential oil (2.1 and 1.5%, respectively) [12].

Trans-pinocamphone was the main compound present in the essential oils of *V. gratissima* after all homeopathic *Phosphorus* treatments, and in the controls, ranging from 28.7 to 34.0% (Table 2). Other marker compounds were *trans*-pinocarvyl acetate (10.1 and 13.4% for 6CH and 12CH, respectively), β-pinene (8.5 and 14.9%, for 9CH and 15CH, respectively), *trans*-pinocarveol (7.4 and 8.8% for 5CH and 12CH, respectively) and *cis*-pinocamphone (6.5 and 7.4% for 27CH and 30CH, respectively) (Table 2).

V. gratissima (*Aloysia gratissima*) harvested in Sao Paulo (Brazil) showed a chemical plasticity since isopinocamphone (25.4%), limonene (15.1%), guaiol (12.7%) and *cis*-pinocarvyl acetate (8.3%) were evidenced as main compounds [14]. These results were in contrast with our work since the percentage of limonene in *V. gratissima* essential oil was too low (from 0.6 to 1.0%) (Table 2).

Plants of Brazilian-lavender from northeast Argentina showed β -elemene (tr to 35.7%), viridiflorol (0.9–33.6%), β-caryophyllene (1.8–28%), α-thujone (6.8–17.5%), 10-*epi*-cubebol (0.1–13.4%), bicyclogermacrene (3.8–12.8%), (E)-nerolidol (tr to 11.6%), and germacrene D (1.9–10.1%) as the principal constituents of the essential oil [15].

V. gratissima plants, grown in northwest Argentina without any agrochemical treatment, showed the presence of 1,8-cineole (45.5%), thymol (17.4%) and sabinene (8.3%) in the essentials oil [16]. In our work, 1,8-cineole (from 0.5 to 0.9%) and sabinene (from 0.1 to 0.3%) were identified in very low amounts after *V. gratissima* homeopathic *Phosphorus* treatments (Table 2). These variations in essential oil composition and percentage of each compound were associated with the ontogeny of the plant population and with the geographical origin of the plant material.

This study demonstrates the ability of homeopathic preparations to positively influence the metabolism of *V. gratissima*, increasing the production of essential oil in comparison with the two controls, one water without the homeopathic energy and the other with the application of ethanol 70°GL (Table 2).

The quali-quantitative composition of *Ocimum basilicum* essential oil after different homeopathic treatments with *Sulphur*, *Calcarea carbonica*, *Carbo vegetalis*, *Silicea*, *Arsenicum album* and *Phosphorus*, but with the same number of dynamizations (30CH), was not influenced [13].

Our study shows that *V. gratissima* has plastic physiological behavior since the applications of dynamized homeopathic *Phosphorus* provided variations in growth, dry weigh, yield of essential oil and essential oil quali-quantitative composition (Table 2). In general, results coming from homeopathic treatments are not linearly related with the dynamization applied, according to the data observed in the literature [17].

The classification of the identified compounds in the essential oils of *V. gratissima* based on functional groups is summarized in Figure 1. Oxygenated monoterpenes predominated, representing 60.4-48.8% of the total volatiles, while oxygenated sesquiterpenes constituted 21.9-14.6% of the oil. The number of indentified compounds ranged from 43.7 (average of three replications) in the control with ethanol 70%, to 31.7 in dynamization 9CH (Table 2). The dynamization *Phosphorus* 9CH showed the highest percentage of oxygenated monoterpenes (60.4%) (Figure 1).

Table 2: Chemical composition of *Verbena gratissima* essential oils obtained by homeopathic *Phosphorus* in comparison with two controls (distilled water and ethanol 70° GL) (average of three replications).

	LRI*	Ethanol	H_20	5CH	6CH	9CH	12CH	15CH	18CH	21CH	24CH	27CH	30CH
α-Pinene	939	0.9 ± 0.01	1.0 ± 0.10	1.5 ± 0.13	1.2 ± 0.19	1.5 ± 0.15	1.7 ± 0.40	0.8 ± 0.07	1.2 ± 0.06	0.8 ± 0.07	1.4 ± 0.07	1.1 ± 0.13	1.0 ± 0.06
Sabinene	976	0.2 ± 0.01	0.2 ± 0.04	0.3 ± 0.01	0.2 ± 0.03	0.3 ± 0.01	0.2 ± 0.08	0.2 ± 0.00	0.3 ± 0.03	0.2 ± 0.05	0.3 ± 0.03	-	0.2 ± 0.02
β-Pinene	980	9.6 ± 0.02	11.0 ± 0.90	14.8 ± 1.26	12.0 ± 1.41	14.9 ± 1.29	12.6 ± 3.20	8.5 ± 0.28	12.1 ± 0.93	9.0 ± 0.50	14.5 ± 0.94	8.9 ± 1.03	10.7 ± 0.60
Myrcene	991	0.1 ± 0.0	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.02	-	0.2 ± 0.00	-	0.1 ± 0.04	-	0.1 ± 0.00	-	0.2 ± 0.03
p-Cymene	1026	0.2 ± 0.03	0.2 ± 0.04	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.01	0.2 ± 0.04	0.2 ± 0.00	0.2 ± 0.01	0.1 ± 0.00	0.2 ± 0.01	-	0.2 ± 0.03
Limonene	1031	0.9 ± 0.02	0.8 ± 0.07	1.0 ± 0.11	0.8 ± 0.09	1.0 ± 0.12	0.9 ± 0.17	0.6 ± 0.09	0.9 ± 0.03	0.6 ± 0.04	0.8 ± 0.05	0.7 ± 0.06	0.8 ± 0.04
1,8-Cineole	1033	0.7 ± 0.01	0.8 ± 0.08	0.9 ± 0.07	0.7 ± 0.04	0.9 ± 0.06	0.9 ± 0.21	0.6 ± 0.04	0.8 ± 0.01	0.7 ± 0.02	1.0 ± 0.04	0.5 ± 0.07	0.7 ± 0.06
cis-Sabinene hydrate	1068	0.3 ± 0.04	0.3 ± 0.02	0.4 ± 0.02	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.03	0.3 ± 0.06	0.3 ± 0.02	0.3 ± 0.04	0.4 ± 0.04	0.2 ± 0.04	0.4 ± 0.03
Linalool	1097	3.7 ± 0.1	4.2 ± 0.07	4.1 ± 0.17	3.9 ± 0.02	4.1 ± 0.20	4.6 ± 0.11	4.0 ± 0.12	4.6 ± 0.18	4.1 ± 0.20	4.6 ± 0.11	4.0 ± 0.18	4.0 ± 0.12
α-Campholenal	1125	-	0.2 ± 0.02	0.2 ± 0.00	0.1 ± 0.05	-	-	0.2 ± 0.07	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.02	-	0.2 ± 0.00
trans-Pinocarveol	1139	7.7 ± 0.15	8.2 ± 0.18	7.4 ± 0.19	7.8 ± 0.15	7.6 ± 0.10	8.8 ± 0.20	7.9 ± 0.20	8.4 ± 0.09	8.2 ± 0.15	7.9 ± 0.08	8.3 ± 0.41	8.1 ± 0.09
trans-p-Menth-2-en-1-ol	1140	0.3 ± 0.01	0.4 ± 0.03	0.4 ± 0.08	0.3 ± 0.08	0.4 ± 0.06	0.5 ± 0.03	0.3 ± 0.04	0.4 ± 0.04	0.3 ± 0.06	0.5 ± 0.00	0.4 ± 0.05	0.3 ± 0.03
cis-Verbenol	1140	2.9 ± 0.07	2.9 ± 0.02	2.5 ± 0.14	2.7 ± 0.10	2.6 ± 0.08	3.0 ± 0.23	2.8 ± 0.11	2.8 ± 0.15	3.0 ± 0.09	2.5 ± 0.07	3.4 ± 0.08	2.8 ± 0.19
trans-Pinocamphone	1160	29.7 ± 0.70	31.9 ± 1.97	30.9 ± 2.02	30.5 ± 0.83	32.5 ± 1.22	31.8 ± 1.55	28.7 ± 0.68	30.4 ± 1.46	32.0 ± 1.05	34.0 ± 1.78	30.3 ± 1.28	30.7 ± 0.89
Pinocarvone	1162	0.5 ± 0.00	0.6 ± 0.22	0.4 ± 0.07	0.6 ± 0.08	-	-	0.5 ± 0.11	0.5 ± 0.00	0.5 ± 0.06	0.5 ± 0.12	0.5 ± 0.00	0.5 ± 0.06
cis-Pinocamphone	1173	7.1 ± 0.02	7.3 ± 0.34	7.1 ± 0.24	7.0 ± 0.14	7.1 ± 0.25	6.8 ± 0.22	6.8 ± 0.09	6.6 ± 0.28	7.2 ± 0.44	7.5 ± 0.40	6.5 ± 0.28	7.4 ± 0.27
p-Cymen-8-ol	1183	0.4 ± 0.01	0.4 ± 0.06	0.3 ± 0.05	0.4 ± 0.03	0.3 ± 0.06	0.5 ± 0.09	0.4 ± 0.05	0.4 ± 0.07	0.4 ± 0.01	0.2 ± 0.03	0.7 ± 0.12	0.4 ± 0.03
α-Terpineol	1189	0.4 ± 0.02	0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.04	0.2 ± 0.06	0.3 ± 0.07	0.3 ± 0.12	0.3 ± 0.06	0.4 ± 0.08	0.2 ± 0.06	0.4 ± 0.14	0.4 ± 0.06
Myrtenol	1193	5.0 ± 0.2	5.4 ± 0.16	4.8 ± 0.22	5.1 ± 0.17	4.3 ± 0.11	5.0 ± 0.25	5.3 ± 0.24	5.1 ± 0.07	5.5 ± 0.08	4.7 ± 0.18	5.5 ± 0.17	5.6 ± 0.17
Verbenone	1204	0.4 ± 0.02	0.3 ± 0.01	0.2 ± 0.05	0.4 ± 0.05	0.3 ± 0.06	0.3 ± 0.06	0.3 ± 0.06	0.4 ± 0.05	0.4 ± 0.03	0.2 ± 0.02	0.4 ± 0.03	0.4 ± 0.08
Carveol	1217	0.4 ± 0.04	0.4 ± 0.01	0.3 ± 0.06	0.4 ± 0.06	0.4 ± 0.09	0.4 ± 0.06	0.4 ± 0.04	0.4 ± 0.06	0.5 ± 0.06	0.2 ± 0.01	0.5 ± 0.14	0.3 ± 0.06
Carvone	1242	0.4 ± 0.05	0.4 ± 0.05	0.3 ± 0.01	0.4 ± 0.04	0.3 ± 0.09	0.3 ± 0.06	0.4 ± 0.06	0.4 ± 0.01	0.4 ± 0.06	0.2 ± 0.02	0.3 ± 0.04	0.4 ± 0.03
Isobornyl acetate	1285	1.0 ± 0.03	1.0 ± 0.01	0.9 ± 0.06	1.1 ± 0.08	0.9 ± 0.10	0.9 ± 0.20	1.1 ± 0.09	1.0 ± 0.05	1.1 ± 0.05	1.1 ± 0.05	1.0 ± 0.13	0.9 ± 0.03
trans-Pinocarvyl acetate	1297	11.6 ± 0.09	12.3 ± 0.24	10.9 ± 0.81	13.4 ± 1.25	10.7 ± 0.81	10.1 ± 1.47	12.5 ± 0.53	11.0 ± 0.17	13.1 ± 0.01	11.5 ± 0.41	11.6 ± 0.48	12.1 ± 0.34
β-Caryophyllene	1418	2.7 ± 0.02	1.3 ± 0.05	1.6 ± 0.30	1.8 ± 0.34	1.7 ± 0.22	1.6 ± 0.44	2.1 ± 0.10	1.8 ± 0.06	2.0 ± 0.09	0.9 ± 0.04	2.3 ± 0.18	1.9 ± 0.13
α-Humulene	1454	0.7 ± 0.03	0.4 ± 0.06	0.5 ± 0.08	0.5 ± 0.09	0.5 ± 0.12	0.4 ± 0.17	0.6 ± 0.01	0.5 ± 0.01	0.6 ± 0.03	0.3 ± 0.02	0.7 ± 0.12	0.5 ± 0.03
Alloaromadendrene	1461	0.2 ± 0.03	0.1 ± 0.00	-	0.2 ± 0.01	0.2 ± 0.00	0.2 ± 0.03	0.2 ± 0.01	0.2 ± 0.03	0.2 ± 0.01	-	0.3 ± 0.01	0.2 ± 0.01
Germacrene D	1480	0.6 ± 0.01	0.3 ± 0.09	0.3 ± 0.12	0.3 ± 0.07	0.4 ± 0.03	0.3 ± 0.09	0.5 ± 0.05	0.5 ± 0.03	0.4 ± 0.04	0.1 ± 0.00	0.5 ± 0.07	0.5 ± 0.06
γ-Cadinene	1513	0.3 ± 0.01	0.2 ± 0.01	-	0.2 ± 0.06	0.3 ± 0.00	0.2 ± 0.04	0.2 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	0.2 ± 0.00	0.3 ± 0.00	-
Germacrene B	1556	1.6 ± 0.12	0.6 ± 0.16	0.9 ± 0.28	0.9 ± 0.27	1.0 ± 0.16	1.0 ± 0.37	1.3 ± 0.13	1.1 ± 0.16	0.9 ± 0.08	0.4 ± 0.02	1.6 ± 0.23	1.0 ± 0.11
(+) Spathulenol	1576	0.9 ± 0.10	0.6 ± 0.21	0.5 ± 0.21	0.7 ± 0.22	0.4 ± 0.08	0.4 ± 0.19	0.8 ± 0.07	0.5 ± 0.11	0.6 ± 0.09	0.2 ± 0.08	0.8 ± 0.18	0.6 ± 0.07
Caryophyllene oxide	1581	3.3 ± 0.04	2.7 ± 0.8	2.6 ± 0.97	3.0 ± 0.84	2.2 ± 0.33	2.6 ± 0.92	3.8 ± 0.16	2.7 ± 0.38	3.3 ± 0.33	1.9 ± 0.16	3.7 ± 0.58	2.9 ± 0.41
Guaiol	1595	2.4 ± 0.09	1.6 ± 0.60	1.7 ± 0.63	1.4 ± 0.14	1.8 ± 0.38	1.8 ± 0.79	3.3 ± 0.17	2.1 ± 0.29	1.7 ± 0.19	0.9 ± 0.10	3.1 ± 0.54	1.7 ± 0.21
Humulene oxide II	1606	0.9 ± 0.2	0.6 ± 0.20	0.5 ± 0.22	0.5 ± 0.05	0.6 ± 0.00	0.7 ± 0.35	1.1 ± 0.14	0.8 ± 0.13	0.7 ± 0.12	0.3 ± 0.08	0.9 ± 0.40	0.6 ± 0.13
Bulnesol	1666	0.6 ± 0.01	0.5 ± 0.40	0.4 ± 0.13	0.3 ± 0.10	0.4 ± 0.12	0.4 ± 0.20	0.9 ± 0.05	0.5 ± 0.12	0.4 ± 0.05	0.2 ± 0.01	0.9 ± 0.21	0.4 ± 0.09
Identified components**		99.9	99.9	99.9	100.0	99.9	100.0	99.9	100.0	99.9	100.0	100.0	99.9

*LRI = linear retention index,** Average of triplicate replications.

According to our results for *V. gratissima*, the essential oil from dynamization 5CH presented more total monoterpenes (76.3%), while the essential oil from dynamization 27CH had a higher concentration of total sesquiterpenes (38.1%) (Figure 1). The pinenes are among the most common monoterpenes produced by plants. These compounds are toxic to bark beetles and their pathogenic fungal symbionts [18]. Probably the high concentration of monoterpenes in *V. gratissima* preserves this plant from pathogenic attack. Moreover, linalool and 1,8-cineole emitted by flowers of Brazilian-lavender were useful as attractants for pollinators, including bees, moths and bats.

Among the identified compounds in *V. gratissima* essential oil (Table 2), 1,8-cineole, limonene, linalool and germacrene-D were reported previously as possessing antimicrobial activity [19]. 1,8-Cineole and camphor act as foliar feeding deterrents to large herbivores such as hares and deer. Also, they may provide a competitive advantage to several angiosperm species as allelopathic agents that inhibit germination of seeds of other species [18]. Thus this work on *V. gratissima* essential oil evidenced a probable action as an allelopathic agent.

Hierarchical cluster analyses (HCA) or a dendrogram is a method in which samples are considered as lying in an n-dimensional space and distances between samples are

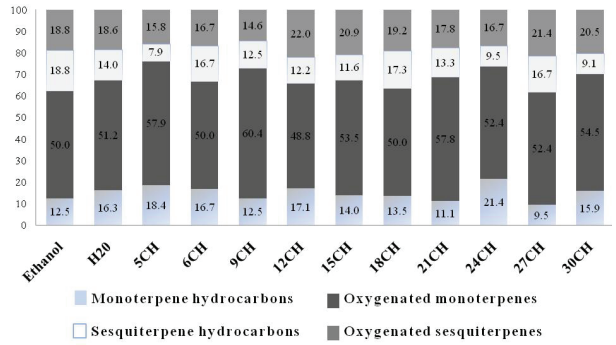

Figure 1: Percentage of compounds in essential oil from 10 dynamizations and two controls (ethanol 70°GL and water) of *V. gratissima* – monoterpene hydrocarbons; oxygenated monoterpenes; sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

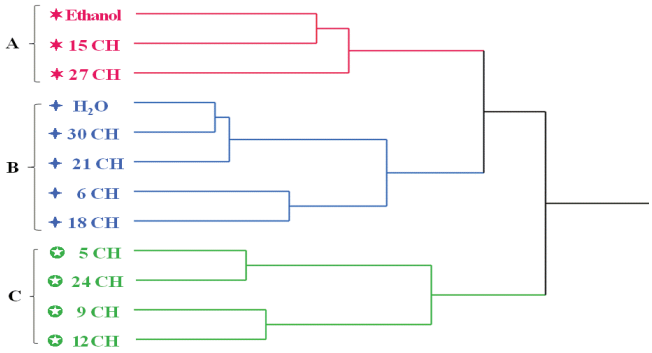

Figure 2: Hierarchical Cluster Analyses obtained from chemical composition of *V. gratissima* essential oils treated with homeopathic *Phosphorus* in comparison with two controls (distilled water and ethanol 70° GL).

calculated joining the objects with an agglomerative procedure [20]. This can be applied to create a hierarchy of clusters of groups with similar data. As observed in the dendrogram, the composition of the essential oils of twelve samples was divided into three major groups: group A (Ethanol 70°GL; 15CH and 27CH), group B (water and 30CH; 21CH; 6CH and 18CH) and group C (5CH and 24CH; 9CH and 12CH) (Figure 2). More similarity was evidenced in group B, in which water and 30CH showed the shortest distance, followed by the dynamization 21CH. Also, group C demonstrated a high hierarchical clustering, while group A had more distance from the others (Figure 2). The similarity observed for the chemical composition of the essential oil is not necessarily in accordance with the growth data. The water control and dynamization 30CH had similar biomass production and yield of essential oil, but were quite distant for evaluation of plant height and diameter of stems (Table 1, Figure 2).

According to the data obtained from the dendrogram, dynamization *Phosphorus* 9CH and 12CH, and insert group C, showed similarity, not only for the essential oil composition, but also for data obtained for growth, biomass production and oil yield (Table 1, Figure 2). Therefore, they could be used for the same purposes in biological agriculture. This use of multivariate statistical analyses for CG-MS data seems to be very useful to investigate and establish the natural correlation within complex studies as with the homeopathic treatment of plants, and HCA permitted a clear indication of the proximities between the different dynamization homeopathic treatments.

Therefore, *V. gratissima* responded specifically to homeopathic preparations, both in vegetative growth, and in the composition of the essential oil, which revealed a good balance between the homeopathic energy, the growth and the plant defense. *Phosphorus* in dynamization 9CH, when applied 3 times a week to *V. gratissima* plants, may interfere with primary and secondary metabolism, showing that the plants treated with homeopathic *Phosphorus* remained healthy, without pest attack and diseases.

Experimental

Plant material: *Verbena gratissima* (Gillies et Hook) Troncoso seeds, collected in March, 2009 from a sample cultivated in a commercial substrate Plantmax® Hortaliças [voucher specimens were deposited at the Herbarium of the Federal University of Lavras (UFLA) under the register number 19810] were cultivated in a greenhouse for 6 months, from April to October, 2009. After 35 days, the plants were transplanted into 10 L pots with a commercial substrate; each treatment was replicated 10 times. Thirty days after transplantation, using randomized and double-blinded experiments, *V. gratissima* was grown with 10 different dynamized homeopathic *Phosphorus* preparations. The homeopathic solutions were applied diluted to 1% in distilled water, in 10 centesimal dynamizations (5CH, 6CH, 9CH, 12CH, 15CH, 18CH, 21CH, 24CH, 27CH and 30CH) in comparison with 2 controls (unsuccussed distilled water and ethanol 70°GL) and administered 100 mL per pot 3 times a week in the morning, for 3 months. The required humidity was maintained.

After 3 months, we evaluated the growth factors: total plant height (shoot, cm), diameter of stem (mm), dry weight of shoot, leaves, root and total (g); root:shoot ratio, leaf weight ratio [leaf weight/ total weight (g g^{-1})] of *V. gratissima* plants. Efficiency of essential oil was calculated using the yield, multiplied by the mean value of the biomass of the dry leaves (mL·plant). A totally randomized design was adopted for the growth analysis, which included 12 different treatments, 10 pots (experimental units) and 2 plants per repetition. Data were analyzed by analysis of variance (one-way ANOVA) and mean values were compared using the Scott-Knott's test ($P < 0.05$).

Isolation procedures: The essential oils from dry leaves of *V. gratissima* (100 g) were extracted by hydrodistillation in a Clevenger type apparatus for 2 h. The essential oils were isolated by weight difference in a centrifuge (1 *g* for 10 min) and the oily phase was separated with the aid of a Pasteur pipette.

Gas chromatography–mass spectrometry: GC–EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures, 220°C and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 30°C/min; carrier gas, helium at 1 mL/min; injection, 0.2 μL (10% *n*-hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data. Moreover, the molecular weights of all the identified substances were confirmed by GC–CIMS, using MeOH as CI ionizing gas. The analyses were performed in triplicate. [21].

Data analysis: Data from essential oil composition for all analyzed samples (homeopathic *Phosphorus* in 10 centesimal dynamizations and the 2 controls) were subjected to hierarchical cluster analyses (HCA) (dendrogram) using the Ward's variance minimizing method [22].

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