



Regrowth of Yerba Mate Plants (*Ilex paraguariensis* A. St. - hill.) Submitted to Dynamized High-dilution Preparations

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Authors' contributions

This work was carried out in collaboration among all authors. Author SD designed the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Author SSW performed the statistical analysis, Author MICB and PB managed the analyses of the study and general orientation. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Yerba mate (*Ilex paraguariensis*) provides leaves and branches as raw material for medicinal and infused / tea drinks, generate employment and income for small farmers and plays an important role in the conservation of the Atlantic Forest. Harvesting causes high stress as a result of drastic defoliation. High dilution preparations have been indicated for treatment of illnesses, also, they showed a positive effect on plant growth. Therefore, the objective of this work was to evaluate the vegetative recovery of yerba mate by using dynamized high-dilution preparations of *Arnica montana*, *Calendula officinalis* and *Carbo vegetabilis* at 12 and 30CH (centesimal hahnemannian), just after formation pruning.

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Place and Duration of Study: The experimental work was conducted in Fraiburgo, SC, Brazil, from July 2015 to August 2016 in agroforestry system.

Study Design: The statistical design was completely randomized with 22 replicates and 7 treatments. Each plant represented an experimental unit and the treatments were applied by spraying.

Methodology: The evaluations started after the first thinning, when number of buds, height and length of regrowth were registered. At 399 days after the first cut, the second cut was performed, and after that, the harvested branches were weighed.

Results: Fresh mass weight was higher in plants treated with *Arnica montana* 30CH (0.2212 kg/plant) and *Calendula officinalis* 30CH (0.2185 kg/plant) in comparison to Potable water (0.1136 kg/plant). Final plant height was 32 cm higher on plants treated with *Calendula officinalis* 30 CH. *Carbo vegetabilis* showed no effects on the regrowth of yerba mate.

Conclusion: Therefore, *Calendula officinalis* 30CH has the potential for use in the recovery of yerba mate plants after harvesting their branches.

Keywords: *Yerba mate*; re-growth; high-dilution preparation.

1. INTRODUCTION

Yerba mate, *Ilex paraguariensis* A. St. Hil., is an arboreal plant species in the Atlantic Forest biome that provides raw material for infused beverages, mainly “chimarrão” (hot) and “tereré” (cold), which are regularly consumed in accordance with local custom in the south of Southern America [1]. Gómez-Juaristi et al. [2] reported the benefits of consuming beverages made from yerba mate, as it contains several bioactive compounds with therapeutic effects on free radicals. Pang et al. [3] reported that consumption of chimarrão and/or tereré helps also to reduce obesity. Pereira et al. [4] found the phenolic compounds presented in yerba mate might aid in the control of diabetes. Antioxidants, which inhibit the proliferation of hepatic and colon cancer cells, are also reported by Mejía et al [5].

In South America, yerba mate plantations cover an approximate area of 540,000 km², overspread among the countries of Argentina, Paraguay, and Brazil [6]. Commercial harvesting of yerba mate is traditionally based on extractivism by cutting branches of naturally occurring plants from forests [7]. As a result of deforestation and occupation of these areas with grain crops and the increased habit of drinking *chimarrão*, yerba mate started to be cultivated as a monoculture [8]. However, the yerba mate cultivated under a monoculture system have presented serious phytosanitary problems. The occurrence and damage of the *Hedypathes betulinus*, for example, are intensified in a monoculture system [9]. In addition, according to Camargo et al. [10], monocultures of yerba mate may lead to a reduction in the content of total phenolic compounds, responsible for the nutraceutical properties of infused beverages.

The design of diversified systems, e.g., Agroforestry Systems, has been proposed in order to optimize the production of yerba mate while maintaining its desired nutraceutical properties [11]. In Agroforestry Systems, there are multiple plant strata that resemble the conditions of the natural environment of occurrence of yerba mate. These conditions with enhanced biotic interactions are conducive of natural processes of ecological regulation [12]. The cultivation of yerba mate, on an agroecological basis, has pleased consumers with healthy foods and added value to the local producers, as farmers have been adopting competitive commercialization strategies [6].

Nevertheless, the raw material extraction method continues to be traditional, with drastic and periodic cuts, even in Agroforestry Systems [13]. The yerba mate plants, when managed incorrectly, are vulnerable to nutrition disorders and physiological disturbances which have a direct impact on productivity [14]. According to the Instituto Brasileiro de Geografia e Estatística - IBGE [15], in the last 25 years, commercial monoculture plantation of yerba mate has been increased its plant density and reduced the interval between harvests. However, productivity continues to decline, and such decrease is estimated to be more than 50% over the next 20 years.

The raw material, leaves and stalks, for chimarrão and tereré, originates from two type of branch harvesting which correspond to the phases of the growing yerba mate plants: (a) performing the arquiteure of the productive mother plant, one to two years after planting and (b) subsequent cycles of annual production [14]. The pruning performing the plant-mother

architecture, breaks the apical dominance and give the better distribution of the main branches to enhance photosynthetic efficiency that results increasing productivity (Budziacki, 2016). However, this practice may causes great initial stress to plants and, hence, it may negatively influence yields [6].

It is recommended that yerba mate pruning must be performed during the vegetative resting time. If pruning is carried out in the growth period, plants may be weakened and end to die as a result [1]. Moreover, stress caused by pruning may be enhanced when inappropriate tools are used; as a consequence, there may be a negative plant response to regrowth and affect the performance of the plants [16]. A poor quality of pruning may also interfere with the return of bud growth and delay the onset and reduce the speed of vegetative regrowth [17]. Because of the extractive character of its cultivation, there has been not so much concern to request research on the management of yerba mate [14].

Studies on growth and physiological development of plants have shown that dynamized high dilutions alter plant metabolism, thus affecting agronomic attributes as well [18]. Bonato and Silva [19] found an increase of radish biomass with Sulfur applications at potencies 5 CH, 12CH, 30CH. Grisa et al. [20] found a productive increase of lettuce with weekly sprays of *A. montana* 6CH after transplanting. It has been demonstrated that chambá (*Justicia pectoralis*) plants treated with *Arnica montana* with dynamization of 100CH (centesimal hahnemannian) increased the level of coumarin (1,2-benzopyrone) [21]. Rossi [18] stated that *Carbo vegetabilis* 100CH provided recovery of strawberry plants, and it could improve vegetative development. Parente et al. [22] reported that *Calendula officinalis* has curative medicinal properties. In agriculture, the use of *Calendula officinalis* up to 30CH is recommended for pruning traumas and for curing injuries in plants [23]. Toledo et al. [24] reported that high-dilution preparations of *Ferrum sulphuricum* and biotherapeutics of tomato leaves at 6CH and 30CH controlled *Alternaria solani* in tomato plants. *Belladonna* 30CH could reduce the foraging activity of cutter ant *Atta* spp. under several conditions [25].

The use of dynamized high dilutions in agriculture takes two approaches: (a) overcoming phytosanitary problems and (b) restoring the dynamic biological equilibrium of organisms in different ecosystems [26]. High-

dilution preparations are considered to be innovative technologies with low cost and minimal environmental impact, used both in phytosanitary management and to guarantee efficiency in healthy food systems [27]. In Brazil, use of high dilution preparations (also called homeopathy or agro-homeopathy) in agriculture is regulated for organic production through Normative Instruction 17 [28]. According to Chikramane et al. [29], dynamized high-dilutions can preserve starting materials and their aggregates, even at extremely high dilutions of 200CH (10^{400}), well above the detectable dilution limit, as determined by Avogadro's constant (6.02×10^{23}). Rao, Roy, Bell [30] reported the use of ultraviolet Raman spectroscopy to identify the differences between high dilutions and pure solvent (control). The study showed that the dynamization process maintains a distinction of physicochemical characteristics.

The objective of this research was to evaluate the influence of dynamized high-dilution preparations on the regrowth of yerba mate plants submitted to pruning under an Agroforestry System.

2. MATERIALS AND METHODS

The study was carried out from July 2015 to August 2016, on a farmer located in the Midwest Region of the State of Santa Catarina, South of Brazil, geographic location at 27°01'43" S and 50°56'32" W (Fig.1). Average precipitation in the experimental area was 1500 mm/year and average annual temperature of 15.3°C [31]. The climate is classified as type Cfb - temperate climate, with mild summer, uniformly distributed rainfall, no dry season, according to the Köppen classification [32]. The agroforestry system was implemented in the winter of 2013, with plants of yerba mate associated with *Maytenus* spp. plants regularly in the planting row, with 5 x 1 m spacing, respectively. The yerba mate rows were spaced at 2.5 m and the distance between plants was 1.5 m, resulting in a density of 2222 plants of yerba mate and 444 plants of *Maytenus* spp., per hectare.

The yerba mate seedlings were purchased at APROMATE-Associação dos Produtores de Erva-mate, located in Machadinho-RS. All the biclonal progeny originated from a progeny of Cambona-4 [33]. In this way, it was possible to establish a common origin of plants and reduce genotypic variability. Seedlings with a height of 15 ± 2 cm were selected for planting. The yerba

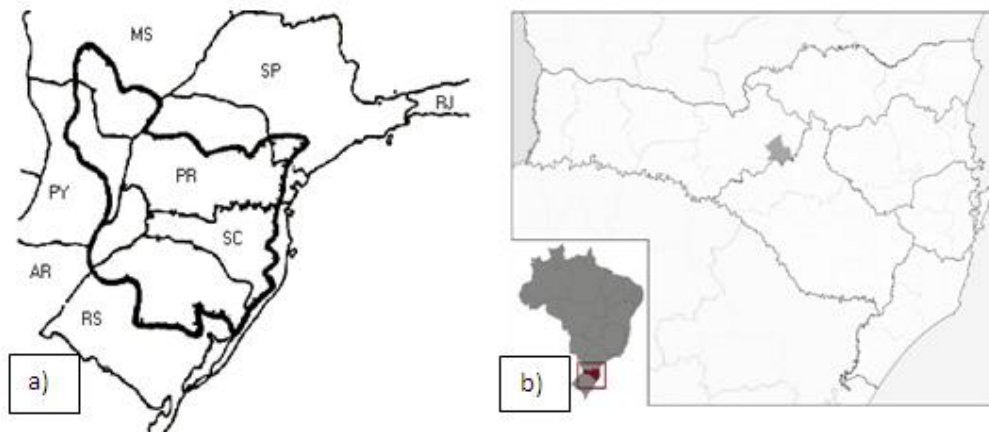


Fig. 1. a) Area of natural occurrence of yerba mate [1] (b) experiments in Fraiburgo, SC, Brazil

mate plants for this study, were those that had more than 50 cm in height and were smaller than 100 cm, identified in sequential numbers, but randomized afterwards, to design the treatments. The experimental design was completely randomized with 7 treatments and 22 replicates each. The experimental unit consisted of one yerba mate plant. Just before to starting the treatments each yerba mate plant was pruned by using a bevel cut, 30 cm from the ground. Each treatment received a code in the Laboratory of Homeopathy and Plant Health of the Experimental Station of Lages / Epagri, hence the experiment was characterized as double-blind.

The dilution preparations *Arnica montana*, *Calendula officinalis* and *Carbo vegetabilis* used in this experiment were selected with the aid of the repertorization process suggested by Gomes [34]. The following repertoire expressions were used: pruning, slow growth, cuts, poor vitality, insufficient vitality and injuries with similar symptoms that could elicit a response from the yerba mate plants. The high dilution matrices were obtained in homeopathic pharmacy. After that in the Laboratory of Homeopathy and Plant Health of the Experimental Station of Lages / Epagri, the high-dilution treatments were obtained by the centesimal hahnemannian dilution method (CH), using the pharmacotechnical process, which consists of successive centesimal dilutions concomitant with 100 vigorous vertical angular agitations [35]. The treatments were: 1) non-chlorinated water, no agitation, as control; 2) *Arnica montana* 12CH; 3) *Arnica montana* 30CH; 4) *Calendula officinalis* 12CH; 5) *Calendula officinalis* 30CH; 6) *Carbo vegetabilis* 12CH; 7) *Carbo vegetabilis* 30CH.

The high-dilution preparations were applied in the proportion of 10 mL L⁻¹ potable water, in a total of one liter per application per treatment. Individual sprayers with a capacity of 1.5 liter were used. In the field, prior to application, the preparation of each treatment was subjected to 100 vigorous shaking through vertical angular movements. The spraying was performed in all shoots of the plant, starting from the top to the point of leaf wetting. The treatments were applied on a monthly basis, at beginning, right after pruning (July 2015) and extended to the end of the experiment (August 2016), in a total of 14 sprays.

The data were collected in a productive cycle of 399 days, from the labelling of plants in the first evaluation until the last pruning. Monthly the length of each experimental yerba mate plant was determined by measuring the vertical distance from the ground surface to the apex of the upper branch of the main stem. Weekly, up to 30 days after starting the treatments the buds and sprout were counted. The length of the shoots and sprouts was also measured weekly by using a electronic digital caliper up to 86 days after the first treatments application. At both pruning period (the first at the beginning and the last at the end of the experiment) the total of fresh cut material (branches with leaves) was weighed with a Master® balance, immediately after been pruned.

Data analysis was performed using software R version 3.4.2 [36], considering 5% of significance. For the weight variable, normal distribution with heterogeneity of variance according to the treatment was considered. For the variables number of buds, shoots up to 10

mm and shoots > 10 mm, the Poisson model was used. For the variables number of shoots up to 50 mm and shoots > 50 mm, the quasi-poisson model was used. The treatments were compared by using the confidence intervals for the mean. For the height variable, the logistic model was used to describe the behaviour over time in days, and the treatments were compared through the model parameters confidence intervals.

3. RESULTS AND DISCUSSION

Fresh weight of the branches measured in the first cut, after homogenization of plant size, did not present differences between plants of the experimental plots before the application of the treatments (Table 1). This results show that there was homogeneity of the plant sampled and selected as experimental plots.

The fresh weight of yerba mate branches, obtained in the 2016 cycle, was higher in the treatments of *Arnica montana* 30CH and *Calendula officinalis* 30CH as compared to

Arnica montana 12CH (Table 2). When taking dilution into account, there was greater weight of the branches in 30CH potencies for the same preparation in comparison to 12CH, in which *Arnica montana* presented significant differences (Table 2). Marques et al. [37] reported accumulation of biomass in *Sida rhombifolia* when treated with the high dilution of *Cymbopogon winterianus* 30CH. In higher dynamizations, the levels of coumarin (1,2-benzopyrone) increased in chambá plants (*Justicia pectoralis*) treated with *Arnica montana* 3CH, 30CH, 60CH and 100CH [21].

Numbers of buds at 30 days after the beginning of sprays and numbers of shoots at 86 days did not present significant differences (Table 3). According to Gomes et al. [34], the impact of the physiological effects of dynamized high-dilution preparations are not Always showed externally impacts, although they may have altered metabolic pathways. Studies have shown that secondary compounds undergo changes when high-dilution preparations are used; for example, the preparations of Silicea 30CH and Sulfur

Table 1. Mean fresh weight of branches pruned from selected yerba mate plants for the composition of the plots, before to start the treatments with the high-dilution preparations accompanied by the respective confidence intervals. Fraiburgo, SC, Brazil, 2015

Treatment	Weight (kg/plant)	Confidence interval	
		Lower limit	Upper limit
Potable water	0.0996 ^{ns}	0.0641	0.1352
<i>Arnica montana</i> 12CH	0.0985	0.0828	0.1142
<i>Arnica montana</i> 30CH	0.1141	0.0870	0.1412
<i>Calendula officinalis</i> 12CH	0.1081	0.0901	0.1261
<i>Calendula officinalis</i> 30CH	0.1115	0.0814	0.1416
<i>Carbo vegetabilis</i> 12CH	0.0973	0.0817	0.1130
<i>Carbo vegetabilis</i> 30CH	0.1085	0.0911	0.1260

ns: not significant by the 5% significance test. CH = centesimal Hahnemannian dilution order

Table 2. Mean of fresh mass of yerba mate branches pruned after application of high-dilution preparations and respective confidence intervals. Homogeneous groups according to the treatment applied and statistical signs. Fraiburgo, SC, Brazil, 2016

Treatment	Estimated value (Kg/plant) ¹	Confidence interval *	
		Lower limit	Upper limit
Potable water	0.1136 ab	0.0753	0.1520
<i>Arnica montana</i> 12CH	0.1075 b	0.0807	0.1344
<i>Arnica montana</i> 30CH	0.2212 a	0.1454	0.2971
<i>Calendula officinalis</i> 12CH	0.1837 ab	0.1177	0.2497
<i>Calendula officinalis</i> 30CH	0.2185 a	0.1483	0.2886
<i>Carbo vegetabilis</i> 12CH	0.1471 ab	0.1045	0.1898
<i>Carbo vegetabilis</i> 30CH	0.1793 ab	0.1195	0.2391

¹ means followed by the same letter do not differ, 5% significance *Intervals obtained with 95% confidence. CH = centesimal hahnemannian dilution order

12CH stimulated the elicitation of peroxidase, catalase, chitinase, -1, 3-glucanase and phytoalexin, which are important compounds for health of cv. Carioca [38].

The yerba mate branches length at the end of the experiment (day 26 of August of 2016, at 399 days after the beginning of the treatments), showed that *Calendula officinalis* 30CH provided the height of 140 cm, about 31 cm longer than those from the plants that were treated only with potable water (Table 4). This treatment differed significantly from *Arnica montana* 12CH, whose estimated mean value of about 36 cm was lower (Table 4). Oliveira et al. [38] concluded that dynamizations of 12CH showed symptoms of phytotoxicity in common bean plants, when compared to dynamization of 30CH. This is indicative of a possible direct relation with the dynamization in use.

The final height of the yerba mate plants treated with *Carbo vegetabilis* (12 and 30CH) did not differ from the height of the plants treated with potable water, *Arnica montana* (30CH) or even *Calendula officinalis* (12CH) (Table 4). Pulido et al. [39] found the lowest growth values of shoots of cabbage with aerial application prepared from *Carbo vegetabilis* at 6CH and 30CH. Increased biomass was found by Rossi et al. [40], whose study reported an increase in shoot size in lettuce plants treated with *Carbo vegetabilis* 100CH, which may suggest the indication of use, at high potencies for this preparation.

Considering the logistic model adjusted for the height variable, there were no significant differences between the treatments for the scale and the x median parameters (Table 5). This occurred because the effect of the high-dilution preparations can only be observed at 200 days after the start of treatments (Table 5). A similar result had been previously found in bud and bud counts, i.e., there were no differences between treatments, either (Table 3). Considering the comparison of treatments by the time interval, the asymptote of the *Calendula officinalis* 30CH treatment differed from potable water and *Arnica montana* 12CH, indicating that there would still be growth effects over time (Table 5). Pulido et al. [39] detected a cumulative effect of the applications in which cabbage plants submitted to treatment with *Arnica montana* 6CH had an increase in biomass, whose significant differences were noted in the final evaluation.

In our studies, *Calendula officinalis* 12CH, *Carbo vegetabilis* 12CH, and *Carbo vegetabilis* 30CH

presented a similar results to those of potable water (Table 5). *Arnica montana* 12CH provided lower final height of the branches and the lowest value for the asymptote, thus reflecting an inhibitory effect on plant growth. However, the plants treated with *Arnica montana* 12CH had a higher number of buds per plant, although, did not show statistical differences (Table 3), which means a positive effect in the stimulation of buds (Santin et al.,2008).

However, after persistent applications with *Arnica montana* 12CH, there are indications that they may negatively influence plant growth (Fig. 1). The application of *Arnica montana* 12CH, at 200 days did not show statistical differences, along with the other treatments (Table 5 - x median). However, after 399 days and by the end of the experiments, there were significant differences from the other treatments (Table 5 - Asymptote). These results may contribute to the creation of more efficient protocols for use of high-dilution preparations, corroborating with the findings of Endler et al. [41], whose study reported that most of the experiments involving high dilutions tend to present measurable results over time.

In the first pruning, which marked the beginning of the treatments, the plants had equal height, and at no point was there an intersection between the treatments (Fig. 1). This is indicative of a continuous cumulative effect in all treatments, with estimated continuity over time. In comparison to the other treatments, the treatment that received *Arnica montana* 12CH had the lowest rate of regrowth acceleration (Fig. 2).

The data in Table 5 and Fig. 2 suggest a non-pathogenetic cumulative effect, unlike the results found by Gomes et al. [34], who stated that pathogenetic experimentation is the best way to conduct experiments with high dilutions. We can state that, during 399 days of treatment, there were no signs of pathogenesis in plants.

In general, there was a cumulative effect as a function of time in the variable plant height, *Arnica montana* 30CH and *Calendula officinalis* 30CH showed greater impact on final plant height. *Arnica montana* 30CH show statistical differences. Pulido et al. [39] found that cabbage plants presented higher vegetative development when treated with *Arnica* 30CH. In our study, plants treated with *Carbo vegetabilis* showed a similar effect to potable water. This tendency is similar to the one found by Pulido et al. [39] for the growth of the shoots of cabbage. In their

Table 3. Mean values of buds and shoots produced by yerba mate plants treated with high-dilution preparations and its respective standard error (SE). No significant differences between the treatments for the variables measured Fraiburgo, SC, Brazil, 2016

Treatment	Buds (no.)		Sprouts up to 10 mm (no.)		Sprouts > 10 mm (no.)		Sprouts up to 0 mm (no.)		Sprouts > 50 mm (no.)	
	Average	SE	Average	SE	Average	SE	Average	SE	Average	SE
Potable water	4.36	0.34	2.00	0.39	2.00	0.29	10.73	0.86	10.73	1.50
<i>Arnica montana</i> 12CH	5.35	0.36	1.61	0.26	2.70	0.40	10.78	0.86	13.13	2.17
<i>Arnica montana</i> 30CH	4.96	0.35	1.61	0.22	2.65	0.27	10.00	1.49	13.43	1.86
<i>Calendula officinalis</i> 12CH	4.70	0.39	1.43	0.19	2.61	0.36	8.13	1.16	12.74	2.11
<i>Calendula officinalis</i> 30CH	5.00	0.31	1.09	0.23	3.22	0.23	11.70	1.08	14.17	1.81
<i>Carbo vegetabilis</i> 12CH	4.48	0.43	1.57	0.25	2.48	0.38	10.39	0.57	12.87	1.75
<i>Carbo vegetabilis</i> 30CH	5.17	0.36	1.57	0.19	2.65	0.34	12.43	1.42	11.26	1.49
Descriptive level (p-value) of the test *	0.7205		0.3699		0.3510		0.1676		0.8477	

* For the variables number of buds, buds up to 10 mm and shoots > 10 mm, the Poisson model was used. The quasi-poisson model was used for the variables number of shoots up to 50mm and shoots > 50mm. Buds at 30 days and number of shoots at 86 days after the start of spraying. CH = centesimal Hahnemannian dilution order

Table 4. Average height of yerba mate plants treated with high-dilution preparations at 399 days after the first pruning. Homogeneous groups according to the treatment applied and statistical signs. Fraiburgo, SC, Brazil, 2016

Treatment	Plant height (cm) ¹	Confidence interval [*]	
		Lower limit	Upper limit
Potable water	109.6818 ab	94.0448	125.3189
<i>Arnica montana</i> 12CH	104.0000 b	88.3629	119.6371
<i>Arnica montana</i> 30CH	131.9130 ab	116.6197	147.2064
<i>Calendula officinalis</i> 12CH	121.7727 ab	106.1357	137.4098
<i>Calendula officinalis</i> 30CH	140.8182 a	125.1811	156.4552
<i>Carbo vegetabilis</i> 12CH	113.8095 ab	97.8045	129.8146
<i>Carbo vegetabilis</i> 30CH	115.8182 ab	100.1811	131.4552

¹ means followed by the same letter do not differ, 5% significance *Intervals obtained with 95% confidence. CH = centesimal hahnemannian dilution order

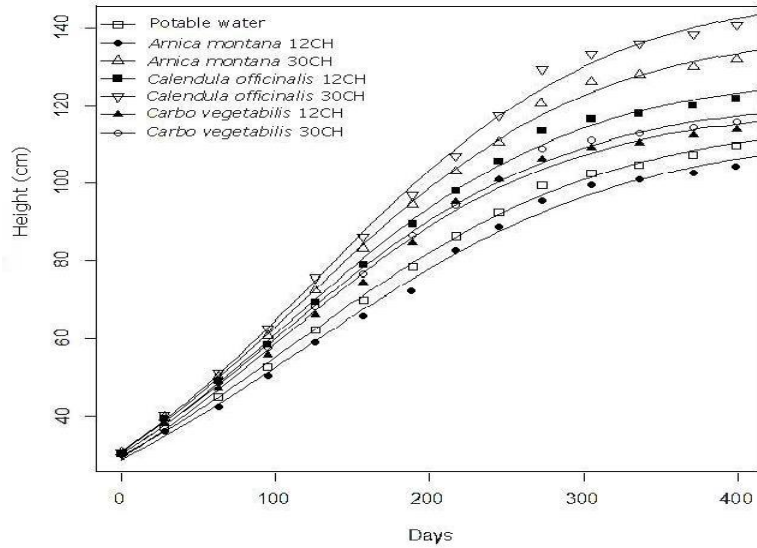


Fig. 2. Average height of yerba mate plants treated with high-dilution preparations. Fraiburgo, SC, Brazil, 2016

Table 5. Estimates of the parameters of the logistic model according to the treatments for the yerba mate plants height variable. Homogeneous groups according to the treatment applied and statistical signs. Fraiburgo, SC, Brazil, 2016

Treatment	Estimated value (cm) ¹	Confidence interval	
		Lower limit	Upper limit
Asymptote			
Potable water	117.26 bc	107.75	133.04
<i>Arnica montana</i> 12CH	113.81 c	106.03	125.44
<i>Arnica montana</i> 30CH	139.96 ab	128.79	157.59
<i>Calendula officinalis</i> 12CH	128.34 bc	118.32	144.00
<i>Calendula officinalis</i> 30CH	150.01 a	138.55	167.41
<i>Carbo vegetabilis</i> 12CH	119.70 bc	111.79	131.11
<i>Carbo vegetabilis</i> 30CH	122.05 bc	112.87	136.26
x median			
Potable water	113.24 a	81.11	136.48
<i>Arnica montana</i> 12CH	117.43 a	88.84	132.42
<i>Arnica montana</i> 30CH	119.19 a	73.06	122.91
<i>Calendula officinalis</i> 12CH	109.61 a	71.27	122.33
<i>Calendula officinalis</i> 30CH	126.49 a	74.66	119.33
<i>Carbo vegetabilis</i> 12CH	104.31 a	73.45	116.23
<i>Carbo vegetabilis</i> 30CH	103.88 a	72.09	122.84
Scale			
Potable water	103.19 a	89.37	149.94
<i>Arnica montana</i> 12CH	107.16 a	97.30	145.96
<i>Arnica montana</i> 30CH	93.22 a	96.36	151.90
<i>Calendula officinalis</i> 12CH	91.74 a	87.01	141.12
<i>Calendula officinalis</i> 30CH	93.13 a	104.88	156.68
<i>Carbo vegetabilis</i> 12CH	91.21 a	85.21	128.95
<i>Carbo vegetabilis</i> 30CH	92.49 a	81.93	134.00

Logistic model coefficients and confidence intervals according to the treatment for height. X median refers to the value obtained in the meantime in days, namely 200 days. ¹ estimated values followed by the same letter do not differ, 5% significance *Intervals obtained with 95% confidence. CH = centesimal Hahnemannian dilution order

study, *Carbo vegetabilis* 6CH and 30CH, was equal effect to the treatment with potable water in relation to height. When subjected to dynamized high dilutions, organisms do not necessarily respond in the same way to external stimuli, and they may respond better to one dynamization than to another [19]. However, Pulido et al. [42] found an increase of fresh and dry mass as well as in height after use of high dilutions on a population of broccoli plants treated with *Sulphur* 6CH and 30CH.

According to the present work, it can be affirmed that dynamized high-dilution preparations can increase the biomass production, and also the size and the canopy of yerba mate plants. This finding shows a cumulative effect on the growth speed of branch height of yerba mate plants over time (Fig. 2).

4. CONCLUSION

High dilution preparations cumulatively impact the height of yerba mate plants. High-dilution preparations of *Arnica montana* and *Calendula officinalis* dynamized at 30CH provided the best results for biomass production, *Arnica montana* 30CH - 0.2212 kg/plant - and *Calendula officinalis* 30CH - 0.2185 kg/plant - in comparison to Potable water (0.1136 kg/plant).

Calendula officinalis 30CH provided the best results for growing (height) of yerba mate plants, final plant height was 32 cm higher on plants treated with Potable Water.

The high dilution preparations of *Arnica montana* at 12CH had a smaller effect on the growth rate of yerba mate plants. The high dilution preparations of *Calendula officinalis* 30CH stimulates the growth rate of yerba mate after thinning, a characteristic that persists over time.

Carbo vegetabilis 12CH and *Carbo vegetabilis* 30CH showed no effects on the regrowth of yerba mate.

Calendula officinalis 30CH has the potential for use in the recovery of yerba mate plants after harvesting their branches.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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