

Effect of fertilization and liming on the content of secondary metabolites in

Hydrocotyle umbellata L. var. *bonariensis* (Lam.) Mr. Spreng

Efeito da adubação e calagem no conteúdo de metabólitos secundários em *Hydrocotyle umbellata* L. var. *bonariensis* (Lam.) Spreng

Efecto de la fertilización y el encalado sobre el contenido de metabolitos secundarios en *Hydrocotyle umbellata* L. var. *bonariensis* (Lam.) Spreng

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Abstract

The nature and the amount of secondary compounds produced by plant species depends on environmental factors, which can act directly on the synthesis of the secondary metabolites. *Hydrocotyle umbellata* L. var. *bonariensis* (Lam.) Mr. Spreng has been traditionally used for medicinal purposes, including for antinociceptive, anti-inflammatory and anxiolytic-like effects, and phytochemical studies revealed its bioactive compounds. This work aimed to evaluate the effects of chemical and organic fertilization, and the soil base saturation correction in the *H. umbellata* crop in dystrophic yellow latosol soil in the production of the secondary metabolites (total phenolic, total flavonoid and hibalactone). The plant was cultivated in the soil of a rural property in the municipality of Anápolis (Goiás state). The experimental design was completely randomized in a controlled environment. The experiment with fertilization had five treatments (control; cattle manure; poultry manure; chemical fertilization; chemical and organic fertilization) and the experiment with liming included the correction of soil base saturation to 20%, 40%, 60% and 80%. The results in most of the two experiments were not statistically significant in the content of the metabolites studied. In the fertilization experiment, the control and manure treatments were statistically better in the content of total phenols in aerial mass analysis. Regarding the saturation correction experiment, the treatment without liming afforded higher levels of hibalactone content (considering the whole plant) and total phenolic content (considering the air mass). The treatment with correction of saturation to 40% afforded higher levels of total phenolic (considering the

whole plant).

Keywords: Chemical and organic fertilization; Total phenolics; Total flavonoids; Hibalactone.

Resumo

A natureza e a quantidade de compostos secundários produzidos pelas espécies vegetais dependem de fatores ambientais, que podem atuar diretamente na síntese dos metabólitos secundários. *Hydrocotyle umbellata* L. var. *bonariensis* (Lam.) Spreng tem sido tradicionalmente usado para fins medicinais, incluindo para efeitos antinociceptivos, antiinflamatórios e ansiolíticos, e estudos fitoquímicos revelaram seus compostos bioativos. Este trabalho teve como objetivo avaliar os efeitos da fertilização química, orgânica e da correção da saturação por bases do solo na cultura de *H. umbellata* em latossolo amarelo distrófico na produção dos metabólitos secundários (fenól total, flavonóide total e hibalactona). A planta foi cultivada no solo de uma propriedade rural no município de Anápolis (GO). O delineamento experimental foi inteiramente casualizado em ambiente controlado. O experimento com adubação teve cinco tratamentos (controle; esterco bovino; esterco aviário; adubação química; adubação química e orgânica) e o experimento com calagem incluiu a correção da saturação por bases do solo para 20%, 40%, 60% e 80%. Os resultados na maioria dos dois experimentos não foram estatisticamente significativos no conteúdo dos metabólitos estudados. No experimento de fertilização, os tratamentos controle e esterco foram estatisticamente melhores no teor de fenóis totais na análise da massa aérea. Em relação ao experimento de correção da saturação, o tratamento sem calagem proporcionou maiores teores de hibalactona (considerando toda a planta) e teor de fenólicos totais (considerando a massa de ar). O tratamento com correção da saturação para 40% proporcionou maiores teores de fenólicos totais (considerando toda a planta).

Palavras-chave: Adubação química e orgânica; Fenóis totais; Flavonóides totais; Hibalactona.

Resumen

La naturaleza y cantidad de compuestos secundarios producidos por especies vegetales dependen de factores ambientales, que pueden actuar directamente en la síntesis de metabolitos secundarios. *Hydrocotyle umbellata* var. *bonariensis* (Lam.) Spreng se ha utilizado tradicionalmente con fines medicinales, incluso para efectos antinociceptivos, antiinflamatorios y ansiolíticos, y los estudios fitoquímicos han revelado sus compuestos bioactivos. Este trabajo tuvo como objetivo evaluar los efectos de la fertilización química y orgánica y la corrección de la saturación de bases del suelo en el cultivo de *H. umbellata* en latosol amarillo distrófico sobre la producción de metabolitos secundarios (fenólico total, flavonoide total e hibalactona). La planta se cultivó en el suelo de una propiedad rural de la ciudad de Anápolis (GO). El diseño experimental fue completamente aleatorio en un ambiente controlado. El experimento de fertilización tuvo cinco tratamientos (control; estiércol de ganado; estiércol de aves de corral; fertilización química; fertilización química y orgánica) y el experimento de encalado incluyó la corrección de la saturación de la base del suelo para 20%, 40%, 60% y 80%. Los resultados en la mayoría de los dos experimentos no fueron estadísticamente significativos en el contenido de los metabolitos estudiados. En el experimento de fertilización, los tratamientos de control y estiércol fueron estadísticamente mejores en el contenido total de fenol en el análisis de masa aérea. En relación al experimento de corrección de saturación, el tratamiento sin encalado proporcionó niveles más altos de hibalactona (considerando toda la planta) y contenido fenólico total (considerando la masa de aire). El tratamiento con corrección de saturación al 40% proporcionó niveles más altos de fenoles totales (considerando toda la planta).

Palabras clave: Fertilización química y orgánica; Fenoles totales; Flavonoides totales; Hibalactona.

1. Introduction

The empirical knowledge about of medicinal plants has been transmitted from the ancient civilizations to the present day, and its uses has become a widespread practice in folk medicine (Soares et al., 2009). However, it is necessary scientific basis to evaluate the efficacy and possible risks of the plants, which involves botanical, chemical, pharmacological and toxicological studies, in addition to the development of appropriate pharmaceutical forms and techniques for quality control, in the case of the commercialization of plants as medicines (Paschoal, 1994).

The demand for phytomedicines have been caused a significant increase for this raw material. However, the market and commercialization of medicinal plants are complex and have peculiarities that make it necessary a detailed knowledge of the processes related to them in order to be successful in the sale of production (Scheffer & Correa Jr, 1998).

The cultivation of medicinal plants when poorly managed can favor plants with low content of bioactive compounds, making their commercialization unfeasible or in the opposite way, increasing the amount of substances considered toxic, making the product harmful and, therefore, of therapeutic use unfeasible (Freire, 2004).

There is little information on the nutritional aspects of native plants and their development in cultivated environments, and it is necessary to define behavior patterns for the optimization of production and future yields, especially for applications in medicinal areas (Martins et al., 1998).

It is known that both internal and external factors can influence the level of production of bioactive compounds from medicinal plants. In general, medicinal plants have a short cycle, rapid growth and are harvested in large quantities, thus requiring nutrient supplementation (Furlan, 1998). The fertilization and liming in traditional crops demonstrate a great productivity gain. The effect of soil saturation correction and chemical and organic fertilization is described in crops such as soybean, tomato, melon and orange.

The species *Hydrocotyle umbellata* L. var. *bonariensis* (Lam.) Mr. Spreng is an herbaceous perennial herb common in tropical regions and its habitat ranges from aquatic to semi-aquatic. This specie has high growth rate and reproductive capacity which enable it to quickly colonize large swaths of many habitats (Heneidy et al., 2019). It has great interest in phytotherapy, especially in Ayurvedic medicine, being recommended due to its antinociceptive, anti-inflammatory and anxiolytic activities (refs). Previous study showed the lignan hibalactone as a chemical marker linked to their activities (Oliveira et al., 2017).

Nogueira (2000) verified that the attack of pests was not verified and the only disease identified so far is rust, caused by the fungus *Uromices* sp, and its attack was observed only in crops conducted inappropriately and without causing great economic damage.

Thus, to provide a greater knowledge of the *H. umbellata* cultivation, this study aimed to evaluate the effects of the chemical and organic fertilization, and the correction of saturation, in dystrophic yellow latosol of the species cultivated in municipality of Anápolis (Goiás state of Brazil), on the content of its secondary metabolites (total phenolic, total flavonoid and hibalactone).

2. Methodology

2.1 Experimental planning:

The experiment started in August 2018, with several soil samplings being carried out and in October 2018 the soil of a rural property (-16.250727 Lat., -48.995298 Long.) with 1,100 m of altitude, in the municipality of Anápolis (Goiás state of Brazil). The soil presented the adequate characteristic for the study of saturation correction, being a dystrophic yellow latosol with base saturation of 6.83%, which was used for the experiments.

Soil saturation was correct in the first half of October. The period of action of limestone was almost three months. From January 2019 it was conducted the fertilization, planting, crop conduction, harvest, evaluation of metabolites and statistical analysis of the data. The culture remained for 94 days in the pots for the experiment with chemical and organic fertilization and 98 days in the vessels for the saturation study.

After cultivation all plants were removed heavy and dried. Moving on to dry matter evaluation, metabolite evaluation, and statistical data analysis. The experiment was carried out in plastic pots of 45 cm x 15 cm x 15 cm, the plots, in which each vessel was a plot. All two experiments were with the treatments in number of five and each treatment had four replications, totaling 20 plots, per experiment.

In each pot, five seedlings were planted, with subsequent choice of three plants per pot, standardizing them. Irrigation was performed when necessary, the crop does not support excess water. The management was performed manually, with weed control through the arranquio and removal of some locusts that appeared in the environment.

The treatments in the fertilization experiment were: a) control (T) without fertilization; b) cattle manure (EB) with 60 m³.ha⁻¹; c) poultry manure (EA) with 20 m³.ha⁻¹; d) chemical fertilization (AQ) (with 100 kg.ha⁻¹ of nitrogen, 180 kg.ha⁻¹ of phosphorus and 50 kg.ha⁻¹ of potassium); e) chemical and organic fertilization (AQO) (EB+EA+AQ). The soil received all

fertilization before planting.

In the experiment of correction of saturation the treatments were: a) without addition of limestone (SC); b) Base saturation correction to 20% (S20); c) Base saturation correction to 40% (S40); d) Base saturation correction to 60% (S60); e) Base saturation correction to 80% (S80). In all plots, chemical fertilization with 100 kg.ha⁻¹ of nitrogen, 180 kg.ha⁻¹ of phosphorus and 50 kg.ha⁻¹ of potassium were used.

All plant development was observed and at the end of cultivation all plants were removed and metabolite production was evaluated. The evaluation of secondary metabolites was performed from the aerial mass, separately from the underground mass. The evaluation process (the analyses of metabolites) was carried out at the Federal University of Goiás, in which the protocol and the study of metabolites are in patent mode by the Institution.

The experimental design was completely randomized, because it is the most suitable for the conditions of conducting this experiment (controlled environment) (Banzato & Kronka, 1989). The results of metabolite contents were submitted to variance analysis, and the doses were compared by the Tukey test, at the level of 5% probability.

2.2 Obtaining the vegetable drug:

The plant material from the cultivated plots was removed from the pots, previously described, washed with running water, dried in a greenhouse with forced air circulation at 40°C and crushed. The plant drug obtained in the form of powder was packed under light and moisture and under refrigeration at -18°C.

2.3 Determination of total phenol and total flavonoid content

Total phenolic content (TPc) was determined, in triplicate, according to Mole & Waterman (1987). For that, 1 mL of extract was dissolved in 5 mL of ethanol 70% in a volumetric flask. Sample's spectrophotometer absorbance was measured at 510 nm. Standard curves were prepared with tannic acid. Total flavonoid content (TFc) was determined, in triplicate, following the method by Rolim et al. (2005). Sample's spectrophotometer absorbance was measured at 361 nm. Standard curves were prepared with rutin.

2.4 Determination of hibalactone content

The hibalactone quantifications were performed by High Performance Liquid Chromatography (HPLC) using the chromatographic conditions established by Oliveira et al. (2019). Hibalactone content (Hc) was determined by comparison with the standard (isolated hibalactone) obtained in our previous work (Oliveira et al., 2019). Stock solutions of the standard were prepared in the range of 10–150 µg mL⁻¹. The mean of the three calibration curves and the equation resulting from the linear regression were used to determine the Hc. The method was validated following the Brazilian National Health Surveillance Agency guidelines (data not shown) (Brasil, 2017).

3. Results and Discussion

The crop harvest of the experiment that evaluated the effect of chemical and organic fertilization on acariçoba crop was carried out at 94 days after planting, and the aerial mass was dried and evaluated, separately from the subterranean mass for the production of secondary metabolites (Hibalactone, Total Flavonoids and Total Phenols).

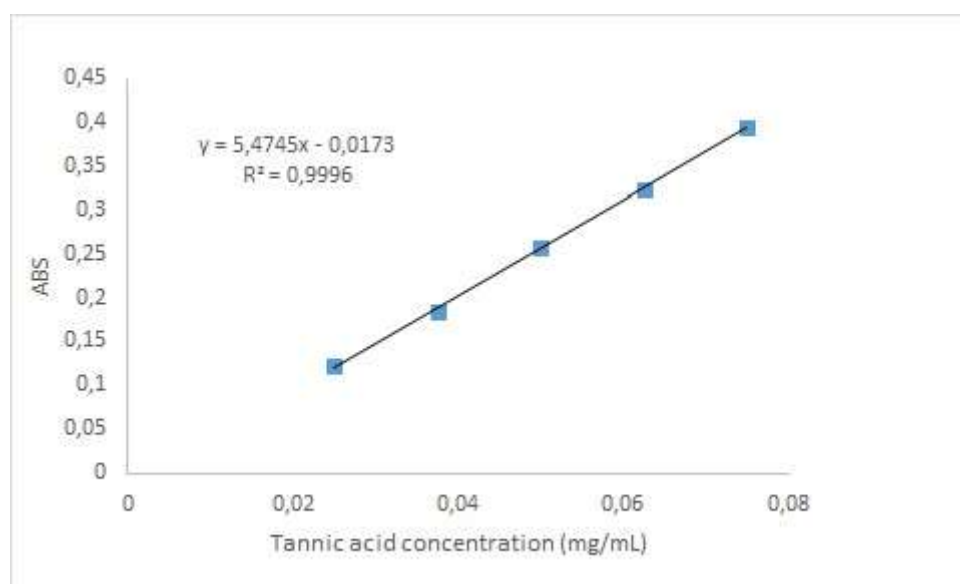
For the quantification of total phenolic compounds, a standard curve of tannic acid was constructed, as shown in Figure 1. From the equation obtained from the standard curve, it was possible to calculate the concentration (mg/mL) and total phenol content of the plant drug.

For the quantification of total flavonoids, a rutin standard curve was constructed, as shown in Figure 2. From the

equation obtained from the standard curve, it was possible to calculate the concentration (mg/mL) and total flavonoid content of the plant drug.

In the analysis of the selectivity of the HPLC method for quantification of hibalactone in the plant drug, the peak corresponding to the hibalactone pattern was observed with retention time of approximately 7 min, which was also verified in the extract. The absorbance spectrum in the ultraviolet region determined for the hibalactone pattern, through the DAD detector, reveals absorbance regions equivalent to the pattern with plant drug extract (Figure 3). The absorption spectra demonstrate that the method is capable of measuring the hibalactone compound in the presence of other constituents, being selective according to the definition of RDC no. 166/2017 (Brasil, 2017).

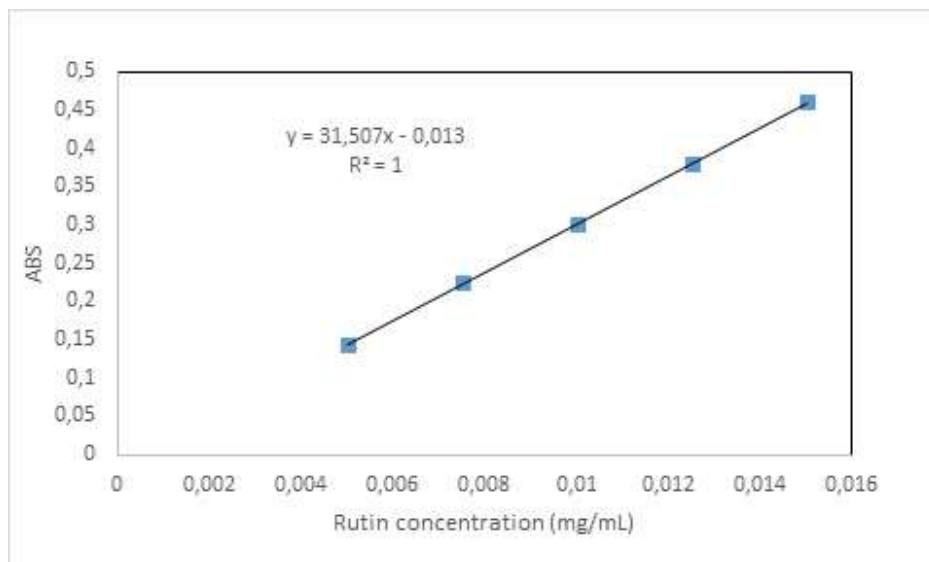
Figure 1. Standard curve for the dosing of total phenols in the plant drug. Tannic acid concentration in mg/mL versus absorbance (ABS). Each point represents the mean \pm standard deviation in triplicate.



Source: Authors.

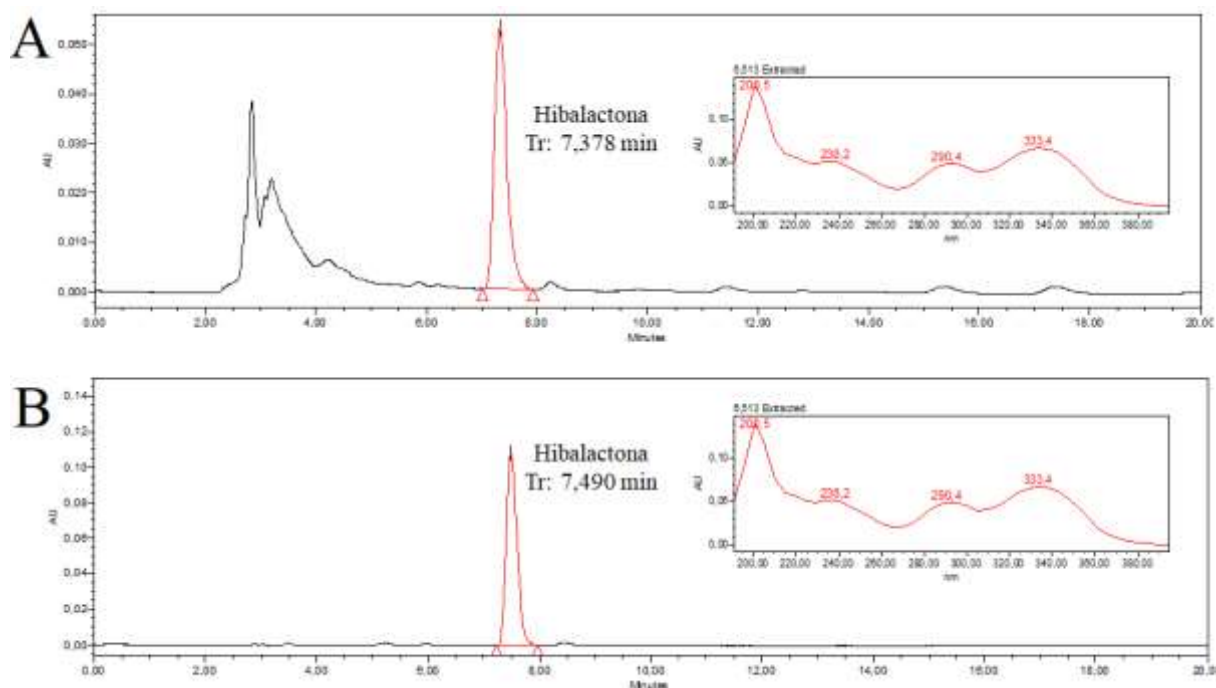
The selectivity for the methanol diluent was also performed by scanning the DAD detector system and did not exhibit maximum absorption that could interfere in the detection of the hibalactone marker at 290 nm according to the co-validated method. Therefore, there was no interference in the chromatograms of the extract or hibalactone pattern.

Figure 2. Standard curve for the dosing of total flavonoids in the plant drug. Rutin concentration in mg/mL versus absorbance (ABS). Each point represents the mean \pm standard deviation in triplicate.



Source: Authors.

Figure 3. Chromatographic profile 2D at 290 nm of plant drug extract (A) and hibilactone pattern (concentration of 0.05 mg/mL) (B) with ultraviolet absorption spectra. Source: Empower 2.0 Program.



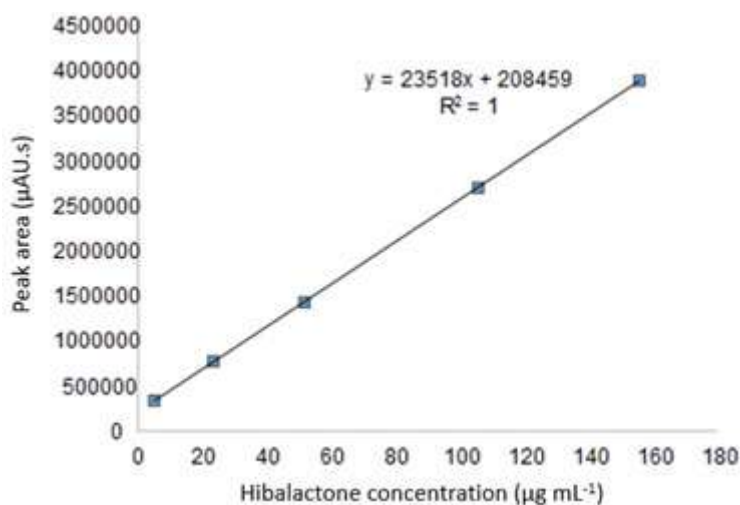
Source: Authors

The calibration curve obtained is shown in Figure 4. The relative standard deviation between the peak areas was less than 5% at all points of the curve. According to RDC No. 166/2017 (BRASIL, 2017), the minimum acceptable value for the correlation coefficient is 0.99. For this attribute the method was considered linear presenting a correlation coefficient equal to 1.0 ($r = 1.0$), which demonstrates that the results obtained are directly proportional to the concentration of the analyte in the sample. The following equation of the line was obtained:

$$y = 23518x + 208459$$

The results that express the precision parameter at the repeatability level are described in tableau 1. For a relative standard deviation limit of no more than 5% (Brasil, 2017), the results showed a relative standard deviation equal to 1.13%, evidencing the reliability of the method developed and validated for quantification of hibalactone in the plant drug.

Figure 4. Hibalactone calibration curve.



Source: Authors.

Table 1 shows the Hibalactone content (in the aerial mass, underground mass and the total of the plant); in Table 2 the total phenol content (in the aerial mass, underground mass and the total of the plant) and Table 3 the total flavonoid content (in the aerial mass, underground mass and the total of the plant) as a function of fertilization (T; EB, EA, AQ and AQO).

Table 1. Data of HPLC analytical method precision at repeatability level for the hibalactone quantification in the sample.

Concentration level	Mass (mg)	Sample concentration (mg/mL)	Area (µAUs)	Hibalactone concentration (mg/mL)	Hibalactone content (%)	RSD (%)
100% (0,02 mg/mL)	1002	40,080	827984	0,0263	0,0657	1,13
	1003	40,140	816020	0,0258	0,0644	
	1002	40,100	829934	0,0264	0,0659	
	1001	40,056	820152	0,0260	0,0649	
	1000	40,032	812906	0,0257	0,0642	
	1001	40,048	815031	0,0258	0,0644	

RSD% = relative standard deviation.

Source: Authors.

Table 2. Hibalactone content (%) in the acariçoba crop as a function of fertilization applied before planting and evaluated at 94 days of cultivation.

Fertilization	Aerial mass	Subterraneous mass	Whole plant
T	0,0325 ns	0,0238 ns	0,0563 ns
EB	0,0307 ns	0,0422 ns	0,0729 ns
EA	0,0383 ns	0,0521 ns	0,0904 ns
AQ	0,0483 ns	0,0658 ns	0,1141 ns
AQO	0,0252 ns	0,0381 ns	0,0633 ns

Where: witness (T); bovine manure (EB); poultry manure (EA); chemical fertilization (AQ) and chemical and organic fertilization (AQO).
Source: Authors.

In Table 2 we have the Hibalactone content for the culture of acariçoba as a function of treatments (chemical and organic fertilization). The means of these treatments did not differ statistically in all results, but the highest hibalactone production in all evaluations was the AQ treatment that used chemical fertilization. Authors such as Blank et al. (2005); Carvalho et al. (2005); Amaral et al. (2008); Maia et al. (2009); Souza et al. (2010); Chagas et al. (2011); Santos et al. (2011); Veloso et al. (2012) and Sodr e et al. (2013); the results were the average of the treatments with fertilization of medicinal plants not significant in the production of Essential Oil and Metabolites, corroborating the result observed in this study.

Table 3. Total Phenol content (%) in the acariçoba crop as a function of fertilization applied before planting and evaluated at 94 days of cultivation.

Fertilization	Aerial mass	Subterraneous mass	Whole plant
T	6,1460 a	0,8486 ns	6,9946 ns
EB	2,4310 c	1,0886 ns	3,5196 ns
EA	6,1110 a	1,0919 ns	7,2029 ns
AQ	5,6719 b	0,8387 ns	6,5106 ns
AQO	2,1910 c	1,2203 ns	3,4113 ns

Where: witness (T); bovine manure (EB); poultry manure (EA); chemical fertilization (AQ) and chemical and organic fertilization (AQO).
Source: Authors.

In Table 3 of mean total phenol content in the culture of acariçoba, we observed that the means of the air mass content are statistically different in the treatments studied and that the Control (without fertilization) and As (fertilization with Poultry Manure) present the best results, differing by the Tukey test to 5% in the means for the other treatments. Other authors such as Ara ujo et al. (2009); Sales et al. (2009) and Santos et al. (2009) also report statistical and significant differences between the average content of Essential Oil in treatments using organic fertilization in medicinal plants.

Also in chart 3 the means of the total phenols content did not differ statistically in relation to the underground mass and the entire plant. The best average for the underground mass was the AQO treatment (chemical and organic fertilization) and for the whole plant the best treatment was the EA treatment (fertilization with Poultry Manure) and after this the T (without fertilization). As in the analysis of hibalactone content, the results are corroborated by the works of the authors Blank et al. (2005); Carvalho et al. (2005); Amaral et al. (2008); Maia et al. (2009); Souza et al. (2010); Chagas et al. (2011); Santos et al. (2011); Veloso et al. (2012) and Sodr e et al. (2013) in which the average sums of treatments with fertilization of medicinal plants were not significant in the production of Essential Oil and Metabolites.

Table 4. Total Flavonoid Content (%) in the acariçoba crop as a function of fertilization applied before planting and evaluated at 94 days of cultivation.

Fertilization	Aerial mass	Subterraneous mass	Whole plant
T	0,9820 ns	0,3156 ns	1,2976 ns
EB	1,0102 ns	0,2010 ns	1,2112 ns
EA	1,4730 ns	0,2347 ns	1,7078 ns
AQ	1,4857 ns	0,2180 ns	1,7037 ns
AQO	1,2437 ns	0,2549 ns	1,4986 ns

Where: witness (T); bovine manure (EB); poultry manure (EA); chemical fertilization (AQ) and chemical and organic fertilization (AQO).
Source: Authors.

Observing chart 4, the total flavonoid content for the acariçoba crop is observed. The treatments did not differ statistically in all results, but the production of Total Flavonoids for the treatments EA (fertilization with Poultry Manure) and AQ (Chemical Fertilizer) were the highest values, but all not statistically different. In the total flavonoid content in the underground mass, the best treatment was T (without fertilization). It is notepoint that the treatments regarding the production of Total Flavonoids were statistically equal as a result equivalent to the authors as Blank et al. (2005); Carvalho et al. (2005); Amaral et al. (2008); Maia et al. (2009); Souza et al. (2010); Chagas et al. (2011); Santos et al. (2011); Veloso et al. (2012) and Sodré et al. (2013); the results were the average of the treatments with fertilization of medicinal plants not significant in the production of Essential Oil and Metabolites.

Table 5 shows the Hibalactone content (in the aerial mass, underground mass and the total of the plant); in Table 6 the total phenol content (in the aerial mass, underground mass and the total of the plant) and Table 7 the total flavonoid content (in the aerial mass, underground mass and the total of the plant) as a function of the correction of saturation applied before planting.

Table 5. Hibalactone content (%) in the acariçoba crop as a function of the correction of saturation applied before planting and evaluated at 94 days of cultivation.

Fertilization	Aerial mass	Subterraneous mass	Whole plant
SC	0,0387 ns	0,0532 ns	0,0919 a
S20	0,0323 ns	0,0394 ns	0,0717 bc
S40	0,0405 ns	0,0355 ns	0,0760 b
S60	0,0280 ns	0,0332 ns	0,0612 c
S80	0,0333 ns	0,0329 ns	0,0662 bc

Where: no lime (SC) addition; Correction of base saturation to 20% (S20); Correction of base saturation to 40% (S40); Correction of base saturation to 60% (S60) and Correction of base saturation to 80% (S80).
Source: Authors.

In Table 5 we have the Hibalactone content for the acariçoba crop as a function of the correction of soil saturation. These means of these treatments did not differ statistically for air mass and ground mass, but the highest values for air mass were treatments SC (without cathe) and S40 (correction of base saturation to 40%) and as for the underground mass the SC treatment was the most responsive.

Authors such as Mascarenhas et al. (1990); Mascarenhas et al. (1996); Faria et al. (2003); Silva et al. (2007); Ayres and Alfaia (2007) and Benedetti et al. (2009) present different results, because they show that the correction of base saturation

increases crop productivity. Results such as Oliveira Junior et al. (2006); Calgaro et al. (2007); Costa et al. (2007) and Souza et al. (2010) as well as this concluded there was no significant difference in the correction of base saturation.

When we analyzed the total production of Hibalactone in the plants, a statistical difference was observed and the best treatment was CS (without correction) different data from all the other. This result differs from the authors Mascarenhas et al. (1990); Mascarenhas et al. (1996); Faria et al. (2003); Silva et al. (2007); Ayres and Alfaia (2007) and Benedetti et al. (2009), because the behavior was partially the opposite to that observed. As for the authors Oliveira Junior et al. (2006); Calgaro et al. (2007); Costa et al. (2007) and Souza et al. (2010) that had as results, average stake of treatments with soil catthem not significant in the production of Essential Oil.

Figure 5 presents the hibalactone regression equation as a function of soil mite treatments and hibalactone production curve in acariçoba crop. The resulting equation is a third-degree polynomial, and the equation that best fit was: $y = -0.0000003x^3 + 0.000006x^2 - 0.0004x + 0.0247$ ($r^2 = 0.7852$);

Figure 5. Regression equation and response curve of total Hibalactone production in the entire plant as a function of soil base saturation.



Source: Authors.

Table 6. Total Phenol content (%) in the acariçoba crop as a function of the correction of saturation applied before planting and evaluated at 94 days of cultivation.

Fertilization	Aerial mass	Subterraneous mass	Whole plant
SC	4,3319 a	0,3612 ns	4,6931 b
S20	3,8335 d	0,9082 ns	4,7418 c
S40	4,1055 b	0,9176 ns	5,0231 a
S60	4,0255 c	0,8602 ns	4,8858 b
S80	1,7327 e	0,7350 ns	2,4677 d

Where: no lime (SC) addition; Correction of base saturation to 20% (S20); Correction of base saturation to 40% (S40); Correction of base saturation to 60% (S60) and Correction of base saturation to 80% (S80).

Source: Authors.

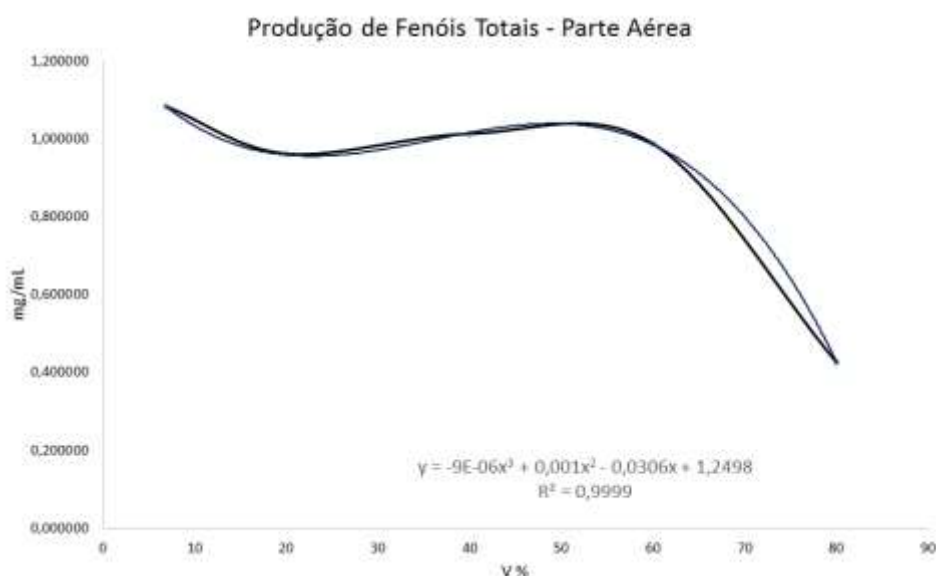
In Table 6, the behavior of the Total Phenols content varies according to the evaluated part of the acariçoba crop. When analyzing the production of phenols in the aerial mass, there was a statistical difference and the best treatment was CS (without correction) being statistically superior to all treatments. This result is different than authors such as Mascarenhas et al. (1990); Mascarenhas et al. (1996); Faria et al. (2003); Silva et al. (2007); Ayres and Alfaia (2007) and Benedetti et al. (2009) because the behavior was the increase with the correction of base saturation and in this work the behavior is the decrease. Being also different from the authors Oliveira Junior et al. (2006); Calgaro et al. (2007); Costa et al. (2007) and Souza et al. (2010) where the treatments were not significant in the production of Essential Oil with the use of soil pantic.

The results of Total Phenol sums when we evaluated the underground mass of Acariçoba had as results different values, the treatment S20 (saturation of 20%) was the highest value, but not statistically significant, corroborated by Oliveira Junior et al. (2006); Calgaro et al. (2007); Costa et al. (2007) and Souza et al. (2010) in which in their cather treatments were also not significant in the production of Essential Oil in the studied crops. Different from the works of Mascarenhas et al. (1990); Mascarenhas et al. (1996); Faria et al. (2003); Silva et al. (2007); Ayres and Alfaia (2007) and Benedetti et al. (2009) which generated an increase in production with soil dredging.

When we evaluated the production of total phenol levels of the whole of Acariçoba we have an interesting behavior, interesting because there was a statistical difference in the treatments having a peak, or rather, a higher and higher value for all when the saturation by soil bases was increased to 40%, corroborated by the authors Mascarenhas et al. (1990); Mascarenhas et al. (1996); Faria et al. (2003); Silva et al. (2007); Ayres and Alfaia (2007) and Benedetti et al. (2009) which the limeing favored and also had in some studies the peak in saturation of 50% as Silva et al. (2007). However, the authors Oliveira Junior et al. (2006); Calgaro et al. (2007); Costa et al. (2007) and Souza et al. (2010) showed statistically non-significant results.

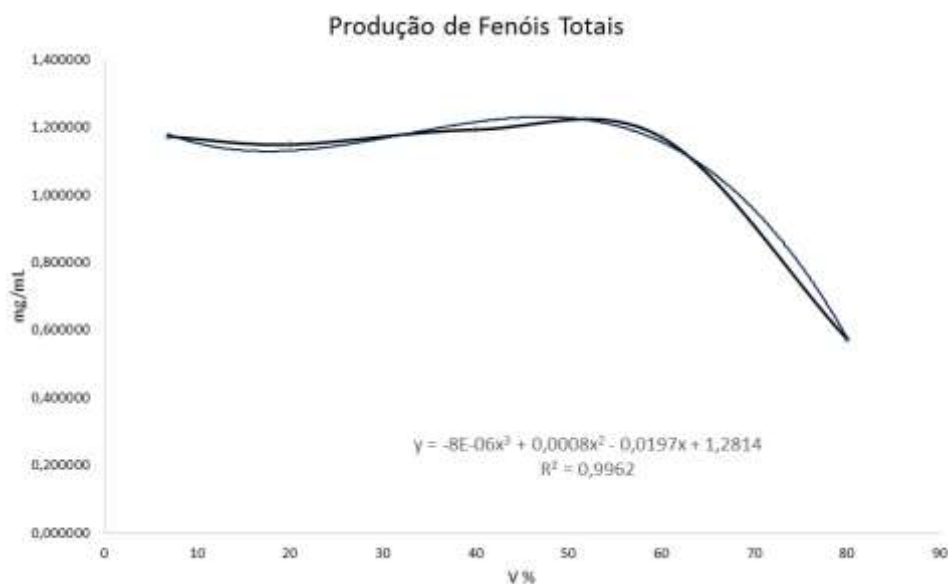
Figures 6 and 7 show the regression equations of the total production of Total Phenols in acariçoba culture as a function of soil base saturation correction treatments and their respective equations, and the one best adapted was third-degree polynomial, with the equations: $y = -0.000009x^3 + 0.001x^2 - 0.0306x + 1.2498$ ($r^2 = 0.9999$), corresponds to the production of the aerial mass of the crop (Figure 6) and $y = -0.000008x^3 + 0.0008x^2 - 0.0197x + 1.2814$ ($r^2 = 0.9962$) corresponding to the production of the entire Acariçoba plant (Figure 7).

Figure 6. Regression equation and response curve of the production Total phenols of the aerial mass as a function of the saturation of soil bases.



Source: Authors.

Figure 7. Regression equation and response curve of total phenol stake in the entire plant as a function of soil base saturation.



Source: Authors.

Table 7. Total Flavonoid Content (%) in the acariçoba crop as a function of the correction of saturation applied before planting and evaluated at 94 days of cultivation.

Fertilization	Aerial mass	Subterraneous mass	Whole plant
SC	1,8510 ns	0,2706 ns	2,1216 ns
S20	1,2803 ns	0,1907 ns	1,4710 ns
S40	1,3296 ns	0,2151 ns	1,5447 ns
S60	1,3143 ns	0,1939 ns	1,5082 ns
S80	1,5164 ns	0,2075 ns	1,7239 ns

Where: no lime (SC) addition; Correction of base saturation to 20% (S20); Correction of base saturation to 40% (S40); Correction of base saturation to 60% (S60) and Correction of base saturation to 80% (S80).

Source: Authors.

Observing chart 7, the total flavonoid content for the acariçoba crop is observed. The treatments did not differ statistically in all results, but the production of Total Flavonoids for the Treatments SC (without correction) were the ones with the highest values. The flavonoid content in the aerial mass, in the underground mass and throughout the plant the best treatment was the CS (without correction), even statistically equal, equivalent to the authors Oliveira Junior et al. (2006); Calgaro et al. (2007); Costa et al. (2007) and Souza et al. (2010) with statistically non-significant results. And disagreeing with the results of the authors Mascarenhas et al. (1990); Mascarenhas et al. (1996); Faria et al. (2003); Silva et al. (2007); Ayres & Alfaia (2007) and Benedetti et al. (2009) which the cathes favored crop production.

4. Conclusion

The two experiments conducted in Dystrophic Yellow Latosol soil with acariçoba culture showed favorable results to the conclusions. The levels of secondary metabolites evaluated showed different behaviors, and some were neither significant, thus we conclude that:

For the experiment with Fertilization:

1. For hibalactone, chemical fertilization provided the highest values of the active ingredient, but not statistically differing from the other treatments;
2. For the total flavonoids all the results were not significant, but as the aerial mass the chemical fertilization provided the highest value, how much the control was the best value and how much the total production of the plant the fertilization with poultry manure was the best;
3. Analyzing the production of total phenols, poultry manure and without fertilization for the levels in the aerial mass of the plant were statistically better than the other treatments. As for the underground mass and the whole plant there is no statistical difference, but the chemical and organic fertilization had the highest value and the poultry manure was the one of the highest value for the whole plant.

For the base saturation correction experiment, we have:

1. For hibalactone, the levels in the aerial mass and underground mass the treatments did not differ statistically, but in the aerial the elevation to 40% saturation and the non-correction for the underground mass were the highest values. As for hibalactone production in the plant, the whole non-correction was statistically higher than the other treatments;
2. For the total phenols of the aerial mass was not significant and higher value with the increase to 40% saturation. As for the underground mass, the non-correction of saturation was statistically superior to the other treatments. When we evaluated the total phenol contents of the plant, the increase to 40% of saturation was statistically the best treatment;
3. For the production of total flavonoids, the treatment without correction of saturation was better than the other treatments and none of the treatments differed statistically.

With the results, there is a need for more studies on Acariçoba, evaluating the effect of chemical and organic fertilization in eutrophic soil; planting density; of the harvest time in the production of secondary metabolites (total phenolics, total flavonoids and hybalactone).

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