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Effects of fertilisation on the initial growth and diversity of secondary metabolites in *Genipa americana*

Efeitos da fertilização no crescimento inicial e diversidade de metabolitos secundários em Genipa americana

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ABSTRACT: The tree species *Genipa americana* ('Jenipapo') is found in the Brazilian Cerrado. It is economically important for its wood and fruits, used in traditional medicine and in the recovery of degraded areas. Based on the multiple uses of this species, we aimed to analyse the effect of different fertilisers (including a control [without fertilisation]) and chemicals (NPK = nitrogen, phosphorus and potassium chloride), both organic (vermicompost) and mixed (NPK + vermicompost), on early field growth and secondary metabolite production in young and mature *G. americana* leaves for 12 months, with monthly collections. The growth analysis indicated that the species develops better in fertilised soils in terms of the height and diameter of the collar, in relation to the control, with the different types of fertilisation presenting equivalent results. Therefore, it is suggested that in the reforestation of degraded areas, such as the Brazilian Cerrado, fertilisation is necessary for 'Jenipapo' planting as an alternative to reduce the degradation levels in this region. The same pattern was found in the analysis of the metabolic classes and the frequency of the treatment compounds, with a predominance of phenolic and flavonoid compounds in young and mature leaves. Only a different pattern was found for NPK + vermicompost, with a greater intensity of tannins and cardiotonic heterosides. The use of leaves for medicinal purposes is advised because, depending on the fertilisation, there may be higher production of cardiotonic heterosides, which are considered toxic.

Keywowrds: Brazilian Cerrado. 'Jenipapo' planting. Polyphenol production. Species for forest restoration.

RESUMO: A espécie arbórea *Genipa americana* ('Jenipapo') é encontrada no Cerrado brasileiro e possui importância econômica, por sua madeira e frutos, além de ser utilizada na medicina tradicional e na recuperação de áreas degradadas. Com base nos múltiplos usos da espécie, objetivou-se analisar o efeito de diferentes adubações, química (NPK), orgânica (vermicomposto) e mista (NPK + vermicomposto), além do controle (sem adubação), no crescimento inicial a campo e na produção de metabólitos secundários em folhas jovens e maduras de *G. americana*. A análise de crescimento indicou que a espécie se desenvolve melhor em solos adubados, possuindo maior altura e diâmetro do colo, em relação à testemunha, com os diferentes tipos de adubação apresentando resultados equivalentes. Logo, sugere-se que nos reflorestamentos de áreas degradas a adubação é necessária para seu melhor crescimento. Os resultados obtidos para as classes metabólicas e frequência dos compostos secundários demonstrou o mesmo padrão, com predominância dos compostos fenólicos e flavonoides, nas folhas jovens e maduras. Apenas NPK + vermicomposto apresentou um padrão diferenciado, com maior intensidade de taninos e heterosídeos cardiotônicos. Entretanto, em seu uso para fins medicinais recomenda-se cautela, pois na dependência da adubação, pode ocorrer maior produção de heterosídeos cardiotônicos, considerados tóxicos.

Palavras-chave: Cerrado brasileiro. Espécies para restauração florestal. Plantio de 'Jenipapo'. Produção de polifenois.

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INTRODUCTION

The species *Genipa americana* L. (Rubiaceae) is a tree that reaches up to 30 meters in height, depending on the region, and it is popularly known as 'Jenipapo,' among other common names in the Amazon, Atlantic Forest, Caatinga, Chaco, and Cerrado biomes. Because it occurs in various vegetation formations, especially in gallery and riparian forests, and it tolerates flooded soils, it is a species that can be used in mixed plantations in swamps and degraded areas of permanent preservation (LORENZI, 2020).

It has economic importance for wood and fruits of commercial value and is a good option for family farmers (CARVALHO, 2003). The species is important in folk medicine, and the fruit has been used against asthma and anaemia (AGRA *et al*., 2008) and to treat jaundice and disorders of the stomach, spleen, and liver, in addition to having antioxidant activity (OMENA *et al*., 2012). The different indigenous Brazilian ethnicities also use this species for medicinal purposes (ELISABETSKY; POSEY, 1989; DELPRETE; SMITH; KLEIN, 2005), and a blue dye is extracted from the fruits and used for body painting, textiles, and ceramics (ALMEIDA, 1993). Due to their diverse uses, most phytochemical and pharmacological studies are performed with fruits, which are mainly composed of iridoids, such as genipin (BENTES *et al*., 2014).

From an ecological point of view, fruits of *G. americana* are consumed by several wild mammals, such as tapirs (*Tapirus terrestris*), white-lipped peccary (*Tayassu pecari*), capybara (*Hydrochoerus hydrochaeris*), and lowland paca (*Cuniculus paca*), in addition to avifauna, such as the toucan (*Ramphastos toco*), which contribute to the natural dispersion of its seeds (DELPRETE; SMITH; KLEIN, 2005; LORENZI, 2020). In addition, the species is recognised as an indicator of the presence of water in the soil, since it prefers flooded soils (LORENZI, 2020).

According to Agra *et al*. (2008), the leaves are used against liver disease in the form of tea, and Nogueira *et al*. (2014) demonstrated their anthelmintic action and antidiarrheal effects. In addition, they are considered forage and used to feed livestock (LORENZI, 2020). Iridoids were also found on the leaves (ALVES *et al*., 2017), in addition to phenolic compounds and derivatives, terpenoids, steroids, alkaloids, and saponins (BESSA *et al*., 2013).

However, the use of this species as an economic resource is still insipient, mainly due to the little information on its growth in the face of different fertilisation conditions. The effect of fertilisation on the production of metabolites is also little known. Thus, the lack of information on the forms of cultivation makes planting by family farmers difficult.

Although there are studies on the response of forest species to the use of nutrients, few studies have been carried out in the field with native species while taking into account the diversity of the Brazilian forest. In this type of study, the collar diameter and height are important for the evaluation of seedling survival and growth potential since the larger diameter indicates a better growth tendency of the roots and, consequently, greater survival capacity.

In addition, studying the effect of fertilisation on the produced phytochemicals is relevant because different edaphic conditions interfere with the production of metabolites of medicinal interest. Vermicompost, the result of the action of earthworms it is widely used since it contains microorganisms and is rich in nutrients, mainly N, Ca, P, Mg, and K, presenting excellent cation exchange capacity and high organic matter content. On the other hand, NPK (nitrogen, phosphorus, and potassium) is the chemical fertiliser most commonly found and used due to its rapid release of ions, made available to plants, and its low cost.

Based on the multiple uses of this tree species that is native to the Brazilian Cerrado, its use in traditional medicine and in the recovery of degraded areas, and considering that the type of fertilisation can interfere with its growth and the presence of secondary metabolites, the objective of this study was to evaluate the effect of different fertilisers on the initial growth in the field and the production of metabolites in young and mature leaves of *Genipa americana*.

2 METHODS

2.1 STUDY SITE

The cultivation of *G. americana* between 2017 and 2018 was conducted at 'Campus Agrárias' (20°26'18.4" S; 54°32'14.6" W), Campo Grande, Mato Grosso do Sul, the central region of the Brazilian Cerrado, in soil classified as Ortic Quartzarenic Neosol. The analysis of the soil indicated: pH = 5.2 (H_2O); phosphorus = 6 mg dm^{-3}; potassium = 36 mg dm^{-3}; calcium = 0.80 cmol$_c$ dm^{-3}; magnesium = 0.50 cmol$_c$ dm^{-3}; H^+ + Al = 3.8 cmol$_c$ dm^{-3}; organic matter = 15.7 g kg^{-1}; cation exchange capacity = 5.2 cmol$_c$ dm^{-3}; base saturation = 27%; clay = 115 g kg^{-1}; silt = 30 g kg^{-1}; and, total sand = 855 g kg^{-1} (EMBRAPA, 2011). Subsequently, the soil was corrected with dolomitic limestone (effective calcium carbonate equivalent – ECCE 90%) in the amount of 1.9 t/ha, with broadcast application, which was incorporated into the soil at a depth of 20 cm 30 days before transplanting the seedlings.

2.2 SAMPLING DESIGN

The seeds used were obtained from 10 matrices in areas of Cerrado in the municipality of Campo Grande and placed in a stainless steel tray with vermiculite, moistened substrate, for germination. After germinating and the seedlings having reached approximately 4 cm in height, they were transplanted to the planting bags and kept in a greenhouse. After preparing the planting area, the plants with 1 year were transplanted in a 30 cm × 30 cm × 30 cm hole, with 3 m × 3 m spacing, and were drip irrigated. The treatments were distributed in the field according to a randomised block design, with four treatments, five replications, and five seedlings per plot.

The treatments were the following: (1) control (without fertilisation); (2) organic fertilisation (vermicompost) with 5 kg per hole (0.027 m^3 of soil) of organic matter, obtained by composting rumen content from cattle by red earthworms from California (*Eisenia foetida* [Savigny 1826]), for 30 days (pH = 7.0; electrical conductivity = 1.2 mS dm^{-1}; P = 260 mg kg^{-1}; K^+ = 600 mg kg^{-1}; Ca^{++} = 25 $cmol_c$ dm^{-3}; H = 6.54 $cmol_c$ dm^{-3}; density = 0.39 g cm^{-3}; and organic matter = 12.9%); (3) chemical fertilisation (NPK= nitrogen, phosphorus and potassium chloride) with 100 g of nitrogen (N), 400 g of phosphorus (P_2O_5), and 75 g of potassium chloride (K_2O) per hole (0.027 m^3 of soil), with simple superphosphate (18% P_2O_5), potassium chloride (60% K_2O), and urea (45% N) used as sources; and (4) mixed fertilisation (NPK + vermicompost) with simple superphosphate, potassium chloride, urea, and vermicompost.

The total amounts of fertiliser were applied following the recommendations for coffee culture (*Coffea arabica* L.) due to the fact that *G. americana* is a Rubiaceae and no specific recommendations for the species can be found. The fertiliser was applied at the bottom of the hole and incorporated into the soil, avoiding initial contact with the roots. The other fertilisers (K and N) were applied around the seedlings every 95 days, each corresponding to 10% of the recommended total, totalling 4 coverings.

2.3 GROWTH ANALYSIS

The collar diameter was evaluated every 30 days for 12 months using a calliper (mm), and the stem and total height (distance from the plant's collar to the highest apex, stem, or leaves) were evaluated with measuring tape (cm). The growth data in terms of height and diameter are presented in the growth curves, with visual growth seen.

2.4 PHYTOCHEMICAL APPROACH

After a year of planting (September, 2019), dozens of young leaves (located at the top of the tree crows) and matures (lower part of the tree crows) were collected separately and dried in a forced air chamber (Marconi®, MA35) at 45°C for 48 hours. Then, they were crushed in an electric mill (Marconi®, MA048) and stored in amber glass bottles, identified, hermetically sealed, and kept in a refrigerator.

The resulting powder was used to obtain the 20% methanolic extract (20 g of the powder and 100 mL of 99.5% methanol) that underwent extraction in an ultrasound bath (Ultrasonic Cleaner®) for 60 minutes, followed by maceration for 48 hours. The pH values of the extracts in all samples were between 5.9 and 6.1.

The extracts were then filtered through a glass and cotton funnel and submitted to phytochemical prospecting through a series of characterisation reactions, evaluating alkaloids, anthocyanins, anthraquinones, phenolic compounds, coumarins, steroids, flavonoids, cardiotonic and cyanogenic heterosides, tannins, and triterpenes (Matos 2009). The analyses were performed in triplicate, and the results were compared with the control sample (20% methanolic extract), observing changes in colour and precipitation. Changes in colour were classified as partial (± = 10%), low (+ = 25%), moderate (++ = 50%), medium (++± = 75%), high intensity (+++ = 100%), or negative (– = 0). The tests with precipitate formation (phenolic compounds and tannins) were carried out in graduated tubes, and the results were classified as partial intensity (<0.2 cm), low (0.21 to 0.5 cm), moderate (0.51 to 0.7 cm), and high intensity (0.71 to 1.0 cm) (FONTOURA *et al*., 2015).

2.5 ANALYSIS OF THE EXTRACTS OF PLANTS BY THIN-LAYER CHROMATOGRAPHY (TLC)

The determination was performed using silica gel GF_{254} chromatoplates with a 8 cm × 7 cm aluminium support (0.2 mm, Merck®). The extracts (10 µL) were applied using a capillary tube, andthe rutin, quercetin, and tannic acid standards (Synth®) were applied in equal volume. As the mobile phase, ethyl acetate/chloroform/methanol/dichloromethane as an eluent (1:1:1:1) and 0.1 ml of acetic acid were used (WAGNER; BLADT, 2009).

To visualise the compounds, nebulisation of oxalic acid/boric acid was performed. Subsequently, the plates were deposited on a heating plate at 100°C for 2 minutes. The visualisation was made through irradiation with ultraviolet light at 254 nm and 365 nm (Vilber Lourma®, VOO-6168), and the determination was carried out by comparing the retention factor (Rf) values of the extracts and standards.

2.6 QUANTIFICATION OF TOTAL PHENOLS AND TOTAL FLAVONOIDS

The phenol content was determined by the Folin-Ciocalteu method using 100 mg of crude extract. The absorbances were measured using a spectrophotometer (Femto®, Model 432) at 750 nm in a quartz cuvette, performed by interpolating the absorbances of the samples using a calibration curve ($y = 0.781x – 0.0031$; $R^2 = 0.9959$) built with gallic acid standard (10 to 300 μg mL^{-1}) with a triplicate design and calculating the average (SOUSA; SILVA; VIERA-JR, 2007).

The quantification of flavonoids was performed using 100 mg of the crude extract of each sample (quercetin as standard), establishing the calibration curve for concentrations of 0.2, 0.3, 0.4, 0.55, 0.7, and 0.8 μg mL^{-1} ($y = 0.132x + 0.0353$ $R^2 = 0.9949$). Absorbances were measured using a spectrophotometer at 425 nm in a quartz cuvette. The experimental design involved performing the measurements in triplicate and the average was calculated (PEIXOTO SOBRINHO *et al*., 2008).

2.7 STATISTICAL ANALYSIS

The data were subjected to analysis of variance (ANOVA), and when there was significance the Tukey test was used at a level of 5% probability, with statistical analyses performed using Assistat software.

3 RESULTS AND DISCUSSION

3.1 INITIAL GROWTH

The results indicated that there were significant statistical differences between the different treatments in relation to the control (Table 1), indicating that the species develops better in the presence of chemicals, organic matter, and chemical/organic fertilisation.

Despite its lower development in soils without fertilisation, at the end of the experiment there was a small variation between the different treatments (<19.4% of the collar and <10.1% of the total height), with the collar diameter being more influenced.

Table 1. Collar diameter, stem height, and total height, *Genipa americana* plants with 12 months of growth submitted to different types of fertilisation

	DF	CV	Control	Vermicompost	NPK	NPK+Vermicompost
Diameter (mm)	3	3.6%	38.0 b	47.0 a	43.1 a	46.6 a
Stem height (cm)	3	2.2%	90.7 b	1084 a	106.9 a	106.5 a
Total height (cm)	3	2.8%	134.7 b	144.8 a	149.9 a	146.4 a

Means followed by same letter in the line do not differ by Tukey test (P>0.05). NKP, nitrogen, phosphorus, and potassium chloride; DF, degrees of freedom; CV, coefficient of variation.

The growth curves (collar diameter and total height) indicated continuous development throughout the evaluation periods. At the end of the evaluated period, the control showed the lowest growth, demonstrating the beneficial effect of fertilisation on the evaluated seedlings (Table 1, Figure 1A–H).

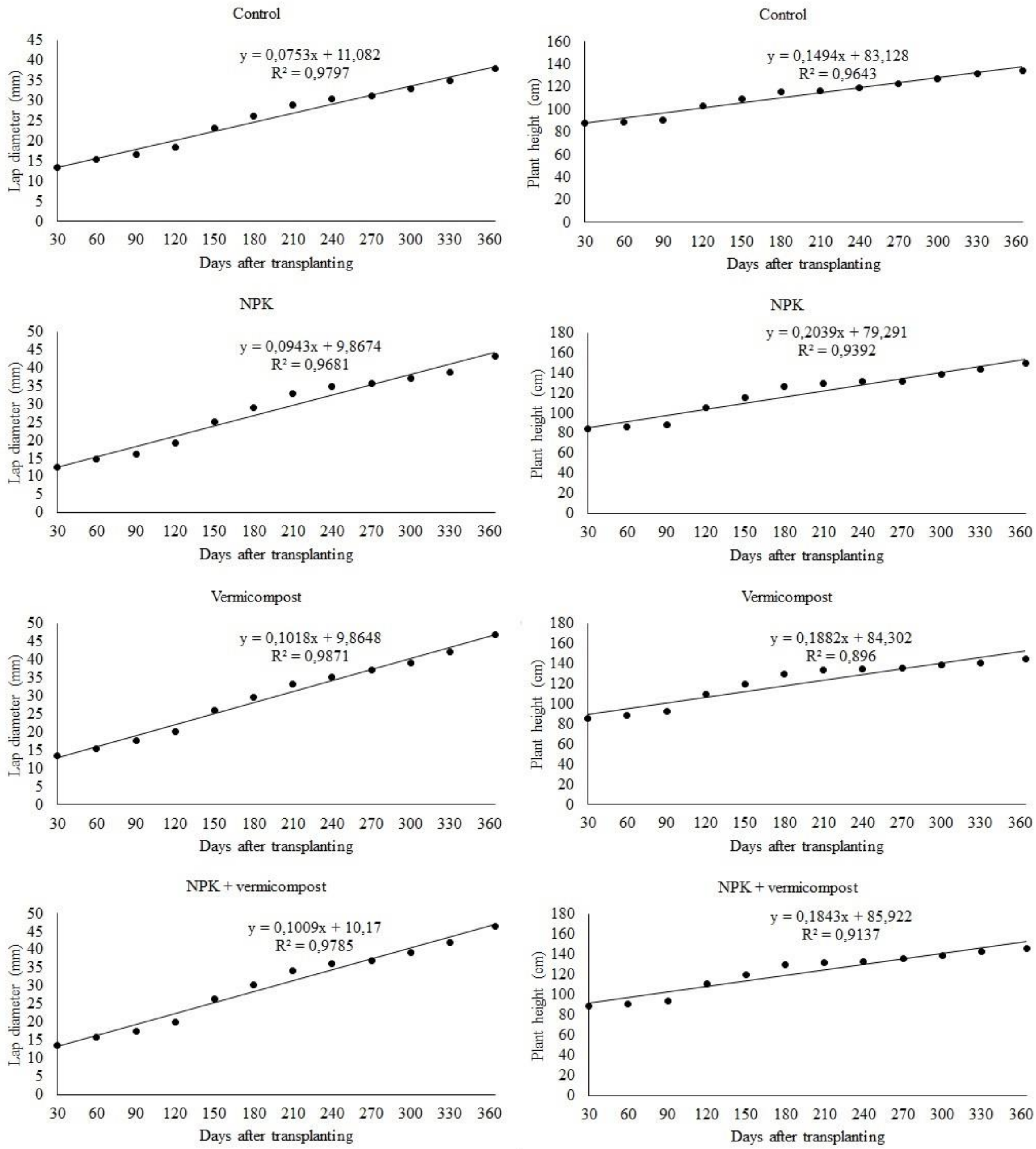

Figure 1. Monthly growth in collar diameter (mm) and total plant height (cm) of *G. americana* plants submitted to different types of fertilisation.

The results indicated better growth of the 'jenipapo' seedlings when adding the different fertilisers, which would be expected since fertilisation of the soil can provide better development. However, the different types of fertilisation can result in different developments, according to the species' ability to absorb the available ions and/or the rate of release of these ions by the fertiliser used. Taking into account the biomes in which the species can be found, adequate growth in different edaphic conditions can be expected, demonstrating its adaptability;

although, less fertile soils, such as the one used in the control, result in a smaller height and diameter.

Valeri, Puerta e Cruz (2003) evaluated the effects of different levels of phosphorus on the initial development of *G. americana*, indicating that there was greater growth in height, diameter, and dry matter production in soils that received higher levels of calcium and organic matter in addition to phosphate fertilisation. These results are similar to those found in this experiment.

The fertilisers used have different characteristics, which can affect plant growth differently. In relation to the vermicompost, the application of organic matter in the soil favours its physical, chemical, and biological characteristics, improving the soil structure and increasing the water retention and aeration capacity, inducing the development of plants. However, the release of ions is slower compared to chemical fertilisers. It must be emphasised that the observed results did not indicate that this factor had a negative influence on the growth of seedlings in the evaluated period.

The NPK enables faster release and greater availability of ions, which could result in rapid absorption of nutrients, making it also suitable for plant growth when compared to the control. The use of chemical fertilizer has proved effective in studies with coffee (*C. arabica* Rubiaceae) (SOUZA *et al*., 2013), where the authors verified an increase in the stem diameter of individuals submitted to different doses of NPK through irrigation and fertigation. In addition, Dubberstein *et al*. (2017) reported an increase in the rate of vegetative growth of coffee plants through mineral fertilisation (N, K, P, Ca, and S).

The combined addition of fertiliser (NPK) with rapid release of ions associated with organic matter (vermicompost), which beneficially alters the physical and chemical characteristics of the soil, could perhaps result in differentiated growth. However, this did not occur during the study period, which could indicate that a longer experimental time would be necessary for differentiation in growth or *G. americana* has a continuous growth rate, adapting to different types of fertilisation, indicating plasticity of adaptation and its potential use in different edaphic environments.

Studies confirming the high phenotypic plasticity of *G. americana*, which was subjected to different environmental conditions, such as natural and artificial shading (LIMA *et al*., 2010), showed a high capacity for adaptation and growth. The species is considered pioneer, early secondary or late secondary, with fast growth and the ability to adapt to different environmental conditions. Its growth characteristics demonstrate its potential to be used as an economic option for small family farmers, for the use of wood and fruits (LORENZI, 2020). For these reasons, promoting the rapid development of plants of commercial and/or pharmacological interest through appropriate edaphic conditions is important for obtaining pharmacologically active substances since the surrounding environment directly interferes with the synthesis of secondary metabolites (YANG *et al*., 2018).

3.2 CHARACTERISATION AND QUANTIFICATION OF SECONDARY METABOLITES

The results of the phytochemical analysis indicated that in young and mature leaves there was the presence of phenolic compounds and flavonoids in high intensity in all treatments. The tannins also occurred at high intensity, but only in mature leaves (NPK + vermicompost treatment) (Figure 2A, B).

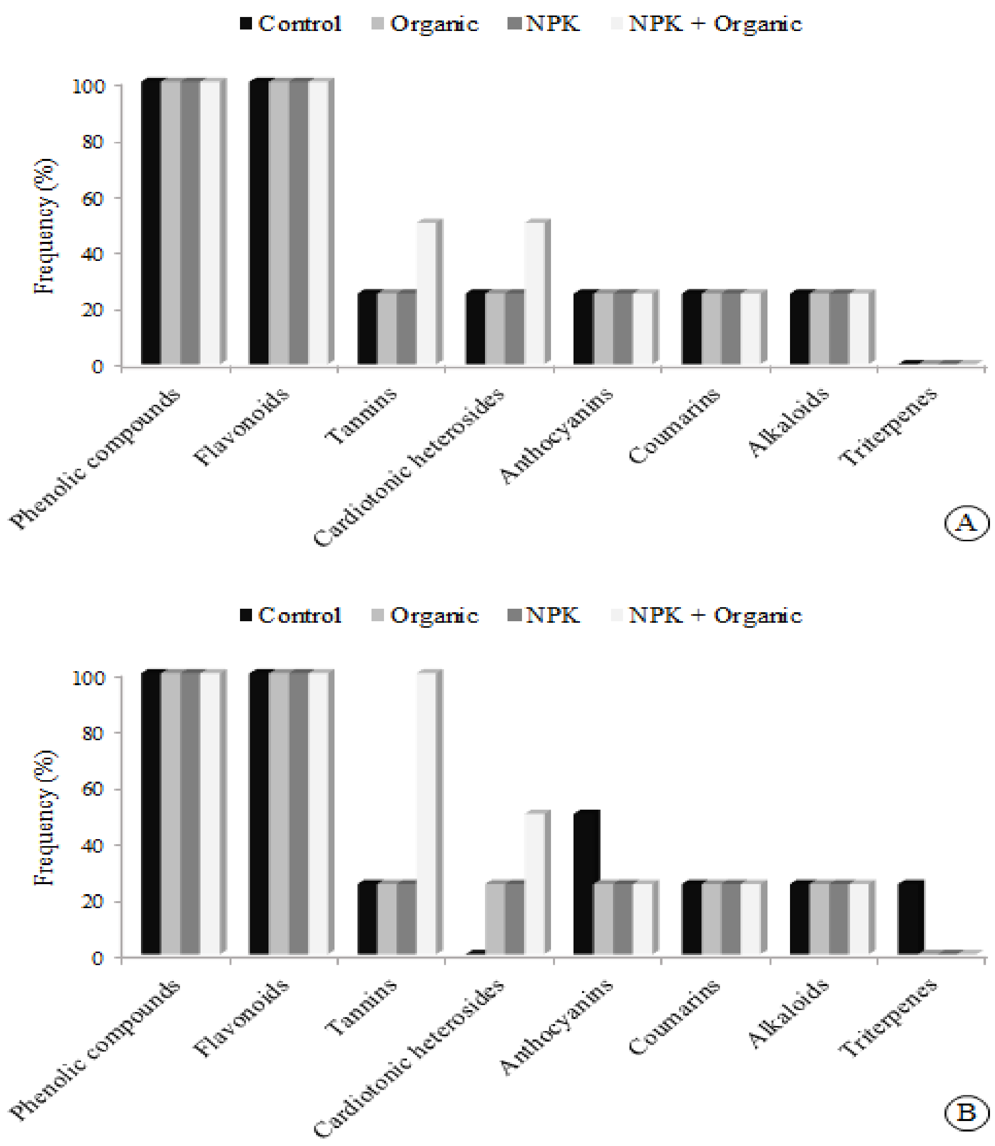

Figure 2. Metabolic classes and frequency (%) of young (A) and mature (B) leaf extracts of *G. americana* plants with 12 months of growth submitted to different types of fertilisation.

At moderate intensity, tannins, cardiotonic heterosides, and young leaves (NPK + vermicompost treatment) were observed. In the mature leaves, cardiotonic heterosides (NPK + vermicompost treatment) and anthocyanins (control) were observed (Figure 2A, B).

At low intensity, coumarins and alkaloids were observed in all treatments for young and mature leaves. At low intensity were also identified, cardiotonic heterosides, young and mature leaves (NPK and vermicompost treatment) and young leaves (control), anthocyanins (in all treatments, except the control, mature leaves), tannins (with the exception of NPK + vermicompost treatment, young and mature leaves), and triterpenes (only on mature leaves, control) (Figure 2A, B).

Thin-layer chromatography analyses indicated fluorescent spots in the quercetin and rutin standards (Figure 3A–D), two types of flavonoids. Its presence was confirmed by the retention factor (Rf quercetin, 0.86; Rf rutin, 0.15), which was similar in all samples. Another major component was observed in the leaves, especially at exposure to 254 nm light (Figure 3B–D); however, its identification was not possible, demonstrating the diversity of the compounds in the species.

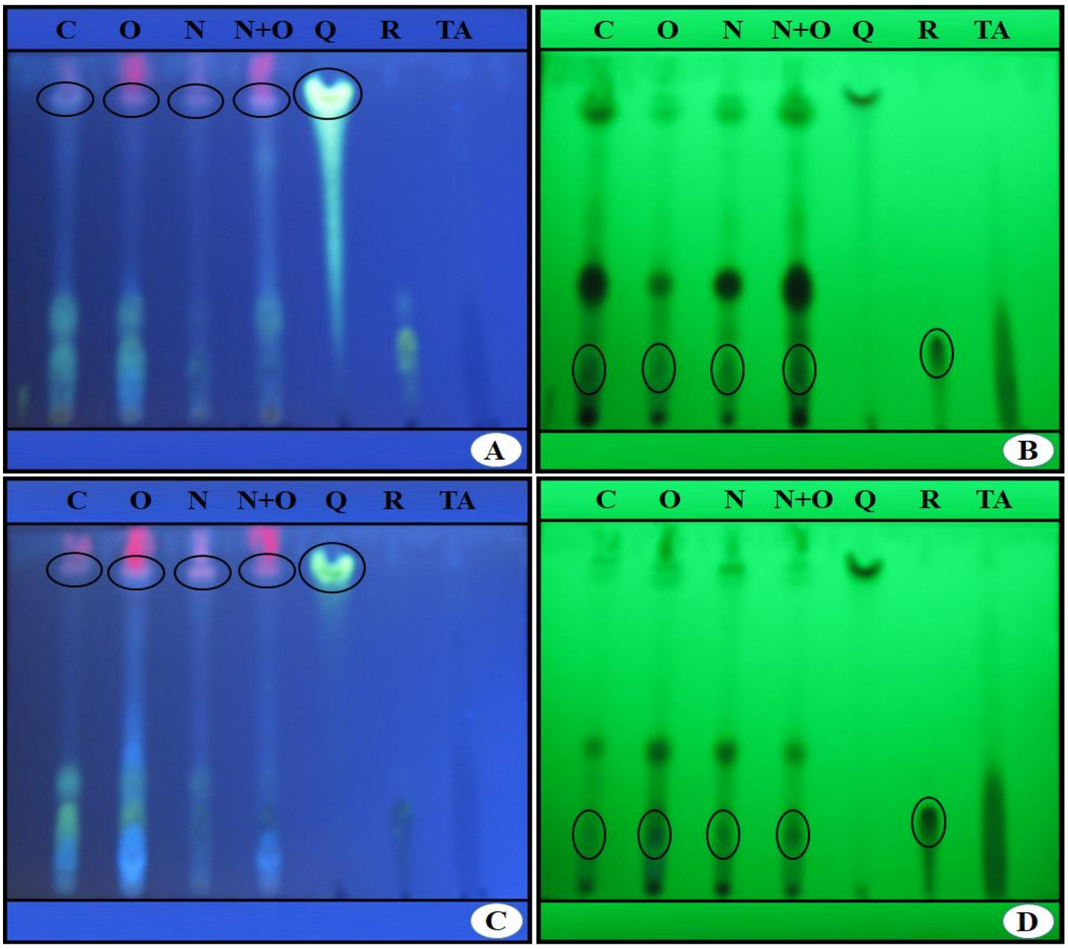

Figure 3. Thin-layer chromatography chromatoplates after revelation and heating under irradiation with ultraviolet light at 365 nm (blue) and 254 nm (green). A–B) mature leaves and C–D) young leaves (C, control; O, organic; N, NPK; N+O, NPK + organic; Q, quercetin; R, routine; TA, tannic acid).

Regarding the measurement of polyphenols (Table 2), there was a higher concentration of total phenols in young leaves, NPK, and control treatments, in relation to the other treatments. On the other hand, the addition of NPK + vermicompost led to a lower production of phenols. Regarding the concentration of total flavonoids (Table 2), the tendency of the mature leaves to have a higher content was observed (control, NPK and mixed treatments).

Table 2. Quantification of phenolic compounds (mg g^{-1}) and flavonoids (mg g^{-1}), young and mature leaves of *Genipa americana* plants with 12 months of growth submitted to different types of fertilisation

	DF	CV	Control	VC	NPK	NPK+VC
Young leaves						
Total phenols	11	2.2%	39.0b	13.0c	42.2a	11.7d
Flavonoids	11	5.2%	1.7a	1.8a	1.9a	1.7a
Mature leaves						
Total phenols	11	3.1%	14.0b	16.4a	7.8d	11.0c
Flavonoids	11	5.0%	2.2b	1.9c	2.1bc	2.5a

Means followed by same letter in the line do not differ by Tukey test (P>0.05). VC = vermicopost; NKP = nitrogen, phosphorus, and potassium chloride; DF, degrees of freedom; CV, coefficient of variation.

The diversity of metabolites found in individuals could be expected, as according to Bessa *et al*. (2013), occurs in the methanolic extract of the leaves of *G. americana* (collected in the state of Tocantins,), tannins, phenols, alkaloids, coumarins, saponins, steroids, and triterpenes. These data are in agreement with the results found in this research, with the exception of steroids (negative result for all tests) and triterpenes, detected only in the extract of mature leaves (control) (Figure 2B).

According to Revilla (2001), the representatives of Rubiaceaes contain anthraquinonesand various types of alkaloids, and in studies with *G. americana*. The author also mentions the presence of mannitol, tannins, methyl ethers, hydantoin, and tannic acids. The results obtained in this research confirm this information, demonstrating the presence of anthraquinones and alkaloids in the leaves.

The main chemical constituents found in the species are attributed several biological activities. Phenolic compounds form a group of substances responsible for producing flavour, odour, and colour in vegetables and they have antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic properties (BABENKO *et al*., 2019).

Within this class are flavonoids, which have antioxidant, antispasmodic, diuretic, and estrogenic properties, among others (PANCHE; DIWAN; CHANDRA, 2016). In plants, they are responsible for colouring, attracting pollinators, and protecting against ultraviolet rays (BABENKO *et al*., 2019). Another important group of metabolites in this class are the tannins, which have bactericidal, molluscicidal, antiviral, and anti-tumour actions (SINGH; KUMAR, 2019).

Alkaloids, a group also found in all treatments (Figure 2A, B), stand out for their relationship with the toxicity and bitterness of plants, influencing their predation, for example. According to Kurek (2019), the alkaloids have antibacterial and antiviral activity, in addition to intensifying the action of antibiotics, among other forms of action.

The cardiotonic heterosides were found in low intensity in almost all treatments (except for mature leaves, control). In medium intensity, in the treatment NPK + vermicompost (Fig. 2A, B). This group is usually related to the toxicity of some plant species, characterised mainly by the action it exerts on the heart muscle. They can be used in the production of drugs, but in high concentrations or constant use, they increase the heart rate, which can lead to damage to the muscle and cause death (SIMÕES *et al*., 2016).

Anthocyanins, found in low intensity in almost all treatments, showed greater intensity only for mature leaves (control) (Figure 2B). This metabolite has antimicrobial, antidiabetic, anti-tumour, and antioxidant properties, retarding of cardiovascular and neurodegenerative diseases (KHOO *et al*., 2017).

Thus, it is suggested that the 'genipapes' found in nature, especially in soils that are poorer in nutrients, such as in the Cerrado, may have their mature leaves used due to the absence of cardiotonic heterosides and a greater presence of anthocyanins. In this sense, popular knowledge regarding the collection of leaves of *G. americana* (mature leaves), is justified by the data found in this work.

The presence and/or intensity of metabolites in a plant can vary according to its location because environmental factors, such as seasonality, temperature, water and nutritional availability, induction by mechanical stimuli and/or attack by pathogens (YANG *et al*., 2018), act in the production and translocation of these compounds. This justifies some variations in the results found in this research, compared to those of Bessa *et al*. (2013).

However, the data obtained in relation to the intensity of the metabolites indicated that the edaphic factors and the age of the leaves had little influence on this factor. Despite the differing concentrations of the nutrients in the substrates, there was a similar production of compounds, again demonstrating the adaptability of the species. The exception was the NPK + vermicompost treatment, which showed higher intensities of cardiotonic heterosides and tannins (young and mature leaves) in relation to the other treatments.

Perhaps some reaction between the organic matter and ions released by NPK or another, unquantified environmental factor led to a different reaction. Thus, using these two types of fertilisers together (NPK + vermicompost) is not recommended, since this could increase the intensity of a metabolite that can cause damage to health. Therefore, use of these fertilisers separately is recommended when the growing conditions are similar to those used in this research.

Pina *et al*. (2018) evaluated the effect of different fertilisers on the production of metabolites in *Moringa oleifera* Lam., demonstrating that the species, when grown on different substrates, presents changes in the chemical composition of its leaves, with differences in the levels of phenolic compounds and flavonoids, in addition to affecting growth. These results demonstrate that the species can react differently to the environmental conditions to which it is submitted, a factor that is probably related to its genetic characteristics and/or regions of origin.

TLC analyses using some flavonoid patterns indicated the presence of quercetin and rutin in young and mature leaves of *G. americana*. According to Omena *et al*. (2012), quercetin is present in the fruits of *G. americana*, being the main flavonoid present in the human diet. It has several pharmacological properties, such as antioxidant and anticarcinogenic potential. Rutin is attributed to its antioxidant action, used against neurodegenerative diseases, among other actions (PANCHE; DIWAN; CHANDRA, 2016). However, there was no mention in the literature of the presence of rutin in this species, but another compound, tannic acid (tannin), despite being mentioned by Revilla (2001), was not confirmed in the chromatographic analysis.

The TLC results again indicated the species' ability to adapt to different edaphic conditions, producing several similar compounds, despite differences in the edaphic environment.

The results, pointing to higher levels of phenolic compounds in young leaves (NPK and Control treatment) may be related to the deviation in nutrients and carbohydrates that occur for the development of these leaves, which, after the fruits, are preferential drains (TAIZ *et al*., 2018). Therefore, in addition to producing nutrients, they receive nutrients exported by mature leaves, which act as a source of resources.

The addition of nutrients to the substrate, in particular nitrogen, can also influence the production of chemical compounds and the synthesis of cellular compounds, such as chlorophyll *(*TAIZ *et al*., 2018), which could explain the best result for NPK treatment in relation to phenolic compounds. With a higher dose of nitrogen in the soil and increased chlorophyll production, the photosynthetic rate increased. Based on this, there may be greater production of carbohydrates and activation of the shikimic acid route, which converts precursors of carbohydrates derived from glycolysis into phenolic compounds (BORGES; AMORIM, 2020). Therefore, in addition to having a fundamental role in plant nutrition, it is suggested that the presence of nitrogen influences the production of phenolic compounds. However, the control, with the lowest concentration of nutrients in the soil, had the second highest concentration of phenols, demonstrating that other factors may interfere with the production of phenolic compounds, in addition to the nutritional availability of the substrate.

The lower production of phenols in NPK + vermicompost fertilisation may be related to two situations: (1) a change in nitrification processes caused by some reaction between NPK and vermicompost, resulting in a lower availability of nitrate, an ion preferably used by plants, resulting in a lower concentration of these metabolites. (2) or because this type of association affects the carbon/nitrogen balance, which directly influenced the primary and secondary metabolism and the translocation of ions to the leaves, leading to a decline in carbohydrate/carbon concentrations and negatively impacting the production of secondary metabolites, which may have affected the production of total phenols, via shikimic acid.

According to Gobbo-Neto and Lopes (2007), there is a well-established correlation between lower levels of phytoconstituents produced under conditions of abundant nitrogen supply and their effects on the levels of shikimic acid derivatives, such as phenolic compounds and polyphenols. However, the same authors state that the relationship between nitrogen fertilization and the production of certain secondary metabolites is not well established, and it is possible to infer that in the initial growth of *Genipa americana* the association NPK + vermicompost negatively influenced the production of total phenols.

The highest levels of total flavonoids, found preferably in mature leaves (control treatments, NPK treatments, and mixed treatments), may indicate greater protection against ultraviolet radiation. Again, the control, despite the edaphic restriction, showed good results in terms of secondary metabolites, demonstrating that the species is adapted to poor and acidic soils.

The results demonstrated that the use of this species in the recovery of degraded areas can occur in soils with or without fertilisation. However, for silviculture purposes in areas of family farming and/or traditional or indigenous communities where leaves are used as part of traditional medicine, care is recommended because, depending on fertilisation, such as with NPK + vermicompost, greater production of cardiotonic heterosides may occur, which are considered toxic.

4 FINAL CONSIDERATIONS

The results indicated that despite the addition of different types of fertilisers, the development was similar in the growth of ‘genipap’ seedlings, indicating the ability to adapt to different types of environments, justifying its wide geographic distribution. In this way, several types of fertilisation can be used in its cultivation.

Regarding the intensity of secondary compounds, the data indicated that there was a high intensity for phenolic and flavonoid compounds; however, fertilisation did not influence the diversity of phytochemicals. The results also demonstrated that the new leaves (NPK and control treatment) are richer in polyphenols and, therefore, more suitable for medicinal purposes. However, the cultivation of the species for use as a medicinal plant must be done with caution since fertilisation with the NPK + vermicompost results in a greater intensity of cardiotonic heterosides, which can be damaging to health.

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