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Cultivation protocol using a coir-based substrate modulates the concentration of bioactive compounds and the antioxidant activity of *Passiflora alata* Curtis seedlings

Protocolo de cultivo à base de pó de coco modula a concentração de compostos bioativos e a atividade antioxidante em mudas de *Passiflora alata* Curtis

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ABSTRACT

The use of coconut coir dust is a low-cost cultivation practice. Thus, this study aimed to determine the effect of coconut coir dust proportion on foliar secondary metabolite biosynthesis and growth of *Passiflora alata* Curtis (sweet passion fruit). This *Passiflora* species possess pharmaceutical relevance and the use of organic-based substrates may promote the production of bioactive compounds in the phytomass. An experiment in a greenhouse was set up with three proportions of coir dust mixed with a commercial substrate for seedlings (CSS) (peat-based) (S1= 1:1:1, CSS: sand: coconut coir dust; S2= 1.5:1.5:1, CSS: sand: coconut coir dust and S3= 1:1, sand: CSS) in seven replicates. After 68 days, growth parameters were measured (height, leaf area, number of leaves, stem diameter, and dry matter), and the harvested leaves were used to prepare ethanolic extracts. The total antioxidant activity and production of phenolics, flavonoids, proanthocyanidins, and saponins were assessed. Unexpectedly, the coir dust supply did not favor the growth and production of secondary metabolites, as the best results were observed in plants grown in S3. Moreover, S3 is efficient in optimizing the growth, metabolite content, and antioxidant capacity of *P. alata* foliar extracts dispensing coir dust supplementation in the substrate. Therefore, adding coir to CSS is not recommended to enhance the production of secondary metabolites and the growth of *P. alata* seedlings.

Index terms: Plant growth; organic residue; secondary compounds; sweet passion fruit.

RESUMO

O uso de pó de coco é uma prática de cultivo de baixo custo. Por isso, o objetivo desta pesquisa foi definir o efeito da proporção de pó de coco para aumentar a biossíntese de metabólitos secundários foliares e o crescimento de *Passiflora alata* Curtis (maracujazeiro-doce). Essa *Passiflora* possui relevância farmacêutica e o uso de substratos orgânicos pode promover o aumento da produção de compostos bioativos na fitomassa. Um experimento foi conduzido com três proporções de pó de coco em substrato comercial para mudas (SCM) (à base de turfa) (S1= 1:1, SCM: areia: pó de coco; S2= 1,5 :1,5:1 , SCM: areia: pó de coco e S3= 1:1, areia: SCM), em sete repetições. Após 68 dias, parâmetros de crescimento foram avaliados (altura, área foliar, número de folhas, diâmetro do caule e matéria seca) e as folhas utilizadas para preparar os extratos etanólicos. Com esses extratos, foi avaliada a atividade antioxidante total e a produção de compostos fenólicos, flavonoides, proantocianidinas e saponinas. A adição de pó de coco e substrato comercial para mudas não favoreceu o crescimento e a produção de metabólitos secundários, pois os melhores resultados foram observados em plantas cultivadas apenas em S3. Além disso, esse mesmo substrato foi eficiente para otimizar o crescimento, os teores de metabólitos e a capacidade antioxidante dos extratos foliares de *P. alata*, dispensando a suplementação de pó de coco no substrato. Portanto, a adição de pó de coco no SCM não é recomendada para aumentar a produção de metabólitos secundários e o crescimento de mudas de *P. alata*.

Termos para indexação: Crescimento vegetal; resíduo orgânico; metabólitos secundários; maracujá-doce.

INTRODUCTION

The market size of herbal medicines was valued at USD 165.66 billion in 2022 and this may grow by 11.16% until 2029 (Fortune Business Insights, 2022). Therefore, the interest to improve the quality of raw materials to boost the productive chain is relevant. In this context, organic substrates to increase the synthesis of bioactive compounds are an alternative that can be utilized in plant cultivation (Machado et al., 2021).

Passiflora species, for instance, have compatibility with organic substrates (Oliveira; Campos; Silva, 2015; Oliveira et al., 2020; Muniz et al., 2022) and a market value of extracts from this genus worth USD 2.69 billion in 2019 (Reports and Data, 2020).

Among low-cost organic substrates, it is possible to highlight the coconut coir dust, which is a fibrous product of *Cocos nucifera* L. mesocarp trituration (Rosa et al., 2001; Oliveira et al., 2020). This residue has conditioner properties, since it improves the soil porosity, presents pH stability and low bulk density, and does not contain phytopathogenic microorganisms (Correa, 2018; Londra; Paraskevopoulou; Psychogiou, 2018). Therefore, this growing media may promote symbiont microorganisms to benefit the plants (Boyer et al., 2016), which may be relevant for the cultivation of *Passiflora* seedlings.

Passiflora alata Curtis is the second most utilized species for formulating anxiolytic passion fruit-based herbal medicines (Fonseca et al., 2020). It occurs due to the presence of bioactive compounds, such as phenols, flavonoids, and tannins, which ensure anxiolytic (Barbosa et al., 2008), sedative (Klein et al., 2014), antioxidant (Ożarowski et al., 2019), gastroprotective (Wasicky et al., 2015), and anticarcinogenic effects (Amaral et al., 2020) in this species. Thus, the optimized biosynthesis of these metabolites can confer more significant medicinal activity to sweet passion fruit phytomass.

The addition of organic matter or specific compounds to the substrate is recommended for plant cultivation (Campos et al., 2015; Souri; Hatamian, 2019). This growing medium should provide water-holding capacity and be affordable (Barrett et al., 2016; Nerlich et al., 2022); such properties are found in coconut coir dust. In other *Passiflora* species, the coconut coir dust was shown to be a viable alternative to enhance the production of foliar secondary metabolites (Oliveira et al., 2020; Muniz et al., 2022; Muniz; Falcão; Silva, 2022; Falcão; Silva, 2022), which is not defined in *P. alata* seedlings.

Therefore, we tested the hypothesis that the application of coconut coir dust to commercial seedling substrate augments the production of bioactive compounds and foliar antioxidant activity in *P. alata* seedlings. The aim of this study was to define the coconut coir dust proportion that increases secondary metabolites biosynthesis, antioxidant activity, and growth of sweet passion fruit seedlings.

MATERIAL AND METHODS

Experimental design

The study was completely randomized, with three proportions of coconut coir dust in seven replicates, totaling 21 experimental units.

Plant material acquisition

The seeds granted by the EMBRAPA *Cerrados* via the PASSITEC Network (Technological Development for Functional and Medicinal Use of Wild Passifloras) were washed in distilled water and mechanically scarified with sandpaper no. 60. Vermiculite (Urimamã Mineração Ltda., Santa Maria da Boa vista, Pernambuco, Brazil) sterilized in an autoclave machine (121 ºC/ 30 min) was used as seed germination substrate and after the emergence of definitive leaves, the plantlets were transferred to seedling bags containing the different substrates tested.

Therefore, three treatments were established: two proportions of coconut coir dust (Viva o Verde®, Recife, Pernambuco, Brazil) mixed with a commercial substrate for seedlings (CSS) that is peat-based (All Garden®, Holambra, São Paulo, Brazil) and sand (Areiasil®, Sirinhaem, Pernambuco, Brazil) and a control substrate (peat-based, without coir dust) were tested, as follows: S1= 1:1:1, CSS: sand: coconut coir dust; S2= 1.5:1.5:1, CSS: sand: coconut coir dust and S3= 1:1, sand: CSS. The chemical characteristics of the substrates are listed in Table 1.

Seedlings were watered daily to maintain 70% of the pore volume filled with water. Thus, they remained for 68 days (October to December 2018) in a greenhouse, with average minimum temperature conditions of 19.7 ºC and a maximum of 38.4 ºC and relative humidity between 67.9% and 68.0%. After this period, the plant material was collected for analysis.

Experiment evaluations

At the end of the experimental conduction, plant growth parameters were evaluated, such as height, with a measuring tape (Starrett®, Pinheiros, São Paulo, Brazil); the stem diameter, using a digital caliper (Lee tools Ltda., Santo André, São Paulo, Brazil); leaf area, by using an electronic leaf area meter (CID Bio-Science Cl 203, Washington, United States) and dry matter of the aerial part, with the assistance of a semi-analytical balance (Marte Científica e Instrumentação Industrial Ltda., São Paulo, São Paulo, Brazil).

Table 1: Characterization of the substrates with different proportions of coconut coir dust used for the cultivation of *Passiflora alata* Curtis.

Substrates	pH	P	Ca^{2+}	Mg^{2+}	Al^{3+}	K^{+}
	(H_2O, 1:2.5)	(mg dm^{-3})			($cmol_c$ dm^{-3})	
S1[†]	5.10	7	1.0	0.50	0.70	0.21
S2[‡]	4.90	9	0.8	0.65	0.85	0.18
S3[§]	4.70	7	0.7	0.50	1.05	0.11

[†]= Sand + commercial substrate for seedlings (CSS) + coconut coir dust (1:1:1); [‡]= Sand + CSS + coconut coir dust (1.5:1.5:1); [§]= Sand + CSS (1:1).

Subsequently, using 500 mg of dried leaves (oven-dried for three days, 45 ºC), extracts were prepared by maceration in 20 mL of ethanol solution (950 mL L^{-1}), in amber flasks, for 12 days (20 ºC) (Química moderna®, Barueri, São Paulo, Brazil) (Oliveira; Campos; Silva, 2015). After this period, the first filtration of the extracts was performed on gauze, and the second on qualitative filter paper. The extracts were stored in a freezer and used for spectrophotometric quantification (Thermo Scientific®, Franklin, Massachusetts, United States) of primary and secondary compounds.

For the dosing of soluble carbohydrates and total proteins the phenol-sulfuric method with the standard curve of sucrose was used, (y= 0.0006x – 0.0605; R^2= 0.9863) (Dubois et al., 1956), and Coomassie blue complexation with the standard curve of bovine serum albumin was used (y= 0.0004x - 0.0149; R^2= 0.9982) (Bradford, 1976).

For phytochemical evaluations, total phenols were quantified using the method described by Orujei, Shabani, and Sharifi-Tehrani (2013), with tannic acid as the standard curve (y= 7.9482x – 0.005; R^2= 0.9871); the total flavonoids were determined by the method suggested by Araújo et al. (2008), of which rutin was used as a standard curve (y= 0.001x – 0.0459; R^2= 0.9952); total saponins were quantified by the method of Vigo, Narita and Marques (2003), using saponin as standard curve (y= 0.0009x + 0.0045; R^2= 0.9926) and total proanthocyanidins were evaluated by the methodology of Queiroz, Morais and Nascimento (2002) and catechin was used as standard curve (y= 0.0412 x + 0.0026, R^2= 0.9926). Details of these analyses have been described by Muniz et al. (2021).

The neutralization capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the plant extracts was evaluated and the results were expressed as the amount of DPPH remaining in the solution to measure the antioxidant activity. DPPH was used to plot a standard curve (y= 0.0245x + 0.0033, R^2= 0.9999) (Rufino et al., 2007).

The analyses were performed using the following reagents: acetic acid (Química moderna®, Barueri, São Paulo, Brazil), sulfuric acid (Química moderna®, Barueri, São Paulo, Brazil; F Maia Ltda., Cotia, São Paulo, Brazil), bovine serum albumin (Sigma-Aldrich®, San Luis, Missouri, United States), catechin (Sigma-Aldrich®, San Luis, Missouri, United States), sodium carbonate (Vetec®, Duque de Caxias, Rio de Janeiro, Brazil), aluminium chloride (Vetec®, Duque de Caxias, Rio de Janeiro, Brazil), cobalt chloride (Nuclear®, Diadema, São Paulo, Brazil), DPPH (Sigma-Aldrich®, San Luis, Missouri, United States), phenol (Vetec®, Duque de Caxias, Rio de Janeiro, Brazil), Folin-Ciocalteau (Merck®, Darmstadt, Hesse, Germany), methanol (Dinâmica Química Contemporânea Ltda., Recife, Pernambuco, Brazil), pyridine (Applichem Panreac®, Darmstadt, Hesse, Germany), rutin (Sigma-Aldrich®, San Luis, Missouri, United States), saponin (Inlab Ltda., São Luiz, Maranhão, Brazil) and acid vanillin (Vetec®, Duque de Caxias, Rio de Janeiro, Brazil).

Data analyses

The obtained results were submitted to analysis of variance (ANOVA) and compared using Tukey's test (p< 0.05) utilizing Assistat 7.7 beta (2016), this same software was chosen to transform the data of saponins using x= √x. Unsupervised machine-learning algorithms were also used to identify profiles that could observe significant differences between the three tested substrates. For this purpose, we applied widely used algorithms for clustering considering all dependent variables. The dependent variables were normalized to avoid distortions in the obtained clusters definitions. The K-means clustering algorithm was run with different values of k, and the best result according to the silhouette metric was k= 3. The original base presented three types of labels, which were not included in the clustering process but were confirmed to have different characteristics.

RESULTS AND DISCUSSION

Contrary to the research hypothesis, the addition of coconut coir dust disfavored the production of secondary metabolites and negatively affected plant growth. Our results demonstrate that the cultivation of this passion fruit in substrates with coconut coir dust is not recommended because of the lower concentration of bioactive compounds and plant growth parameters that were reported (Table 2 and 3), even though the addition of organic substrates is recommended for the production of *P. alata* seedlings (Braga; Junqueira, 2003). Similarly, Hongpakdee and Ruamrungsri (2017) verified that a supply of 75% of coconut coir dust reduced plant growth in *Petunia* spp. hybrids.

The algorithm analysis showed that the result with two clusters indicated that the first two substrates (S1 and S2) had a closer response than the third substrate (S3). We deployed another algorithm to confirm the obtained result, using the process of agglutination as a reference. In this case, we obtained a dendrogram (Figure 1) in which samples 8 to 11 (S3) were separated from samples 0-3 (S1) and 4-7 (S2), ensuring that there is a significant difference between the two first substrates in comparison the to the third considered substrates (Figure 1). Phenols, antioxidant activity, proteins, and carbohydrates were the most relevant components in the analysis (Figure 2).

As mentioned above, the production of secondary metabolites was not enhanced in seedlings grown with coconut coir dust (Table 2) as the highest concentrations of flavonoids and saponins were obtained in S3 (without coir dust), which presented increments of 116.26% and 357.14%, in comparison to plants cultivated with 1/3 of coconut coir dust added (S1). This behavior contrasts with the results found by Gavrić et al. (2021), as the application of mineral fertilizer increased the biosynthesis of essential oils in *Ocimum basilicum* L., which highlights the need for testing a combination of a peat-based substrate (CSS) and mineral fertilizers to determine if there is a synergism to enhance the concentration of bioactive compounds in *P. alata*.

The negative effect of the coir dust on the growth and the production of biomolecules in *P. alata* may be related to the salinity of this residue (Van Gerrewey et al., 2020); likely the washing to remove excess salts before marketing the product may not have been enough, which may result in high oxidative stress in plants (Isayenkov; Maathuis, 2019). However, to confirm this speculation, studies with salinity levels in the substrate must be conducted.

Moreover, under physiological stress, plants may present compromised metabolism (Rahneshan; Nasibi; Moghadam, 2018), similar to plants kept in substrates with the highest proportion of coconut coir dust. This is consistent with the observation of Zuo et al. (2020), in which the cultivation using coir dust caused impaired regulation of genes related to flavonoid biosynthesis in *Dendrobium officinale* Kimura et Migo, in comparison to the pinus-based substrate. On the other hand, there are reports of better antioxidant activity in plants grown in organic substrates than in control plants without fertilizers, but this response depends on the radical tested (Mahmud; Abdullah; Yaacob, 2020). Średnicka-Tober et al. (2020) documented that organic cultivation optimized the production of antioxidant compounds, such as phenolic acids, in *Malus domestica* Borkh, in comparison to conventional cultivation without organic fertilizers.

Table 2: Concentrations (mg g plant^{-1}) of total flavonoids, total phenols, total proanthocyanidins, total saponins (µg g plant^{-1}), total proteins, total soluble carbohydrates, and antioxidant activity (mg g^{-1} remaining DPPH) in *Passiflora alata* Curtis grown in different proportions of coconut coir dust, after 68 days in a greenhouse.

Variables	Substrates			
	S1†	S2‡	S3§	CV (%)
Total flavonoids	271.70b	343.76b	587.58a	17.22
Total phenols	57.37b	34.63c	74.48a	15.14
Antioxidant activity	102.06a	55.86b	30.07b	30.36
Total proanthocyanidins	7.09b	3.50b	40.79a	32.15
Total saponins	0.35b	0.18b	1.16a	9.85
Total proteins	456.44a	221.02b	570.17a	20.03
Soluble carbohydrates	15.36a	9.25b	18.33a	17.95

Mean values (n =7) followed by the same letter do not differ according to Tukey's test (p <0.05). CV= coefficient of variation; †= Sand + commercial substrate for seedlings (CSS) + coconut coir dust (1:1:1); ‡= Sand + CSS + coconut coir dust (1.5:1.5:1); §= Sand + CSS (1:1).

Similarly, the optimal growth of seedlings was registered in plants from S3, considering all the evaluated parameters, compared to the other substrates tested (Table 3). These results are relevant because, in the same treatment, the highest concentrations of other metabolites, such as proanthocyanidins and total phenols were observed (Table 2).

Thus, the proanthocyanidins and phenolics concentrations in *P. alata* seedlings cultivated in S3 were increased by 1.065% and 115.07%, respectively, compared to those plants cultivated in S2 (Table 2), which were positively correlated with the growth parameters that were evaluated (Figure 3). Apart from the other compounds, these were positively correlated with the production of protein and carbohydrates (Figure 3).

As reported in this study, the increase in the concentration of secondary metabolites may be related to the reduction of free radicals present in the extract. These effects can be linked to the compounds evaluated, which have hydroxyls (Zeb, 2020) capable of stabilizing free radicals. These plant antioxidant metabolites can mitigate damage caused by chronic diseases (Zhang et al., 2015).

Table 3: Height (cm), leaf area (cm^2), stem diameter (mm), number of leaves, and dry matter of the aerial part (DMAP) (g) of *Passiflora alata* Curtis seedlings kept in different proportions of coconut coir dust, after 68 days in a greenhouse.

Variables	Substrates			
	S1[†]	S2[‡]	S3[§]	CV (%)
Height	2.82c	3.47b	6.77a	5.09
Leaf area	5.69b	5.37b	120.14a	22.83
Stem diameter	1.61c	1.93b	2.80a	3.27
Number of leaves	4.00c	6.00b	9.00a	11.47
DMAP	0.13c	0.27b	0.53a	20.31

Mean values (n =7) followed by the same letter do not differ by Tukey's Test (p <0.05). CV= coefficient of variation; [†]= Sand + commercial substrate for seedlings (CSS) + coconut coir dust (1:1:1); [‡]= Sand + CSS + coconut coir dust (1.5:1.5:1); [§]= Sand + CSS (1:1).

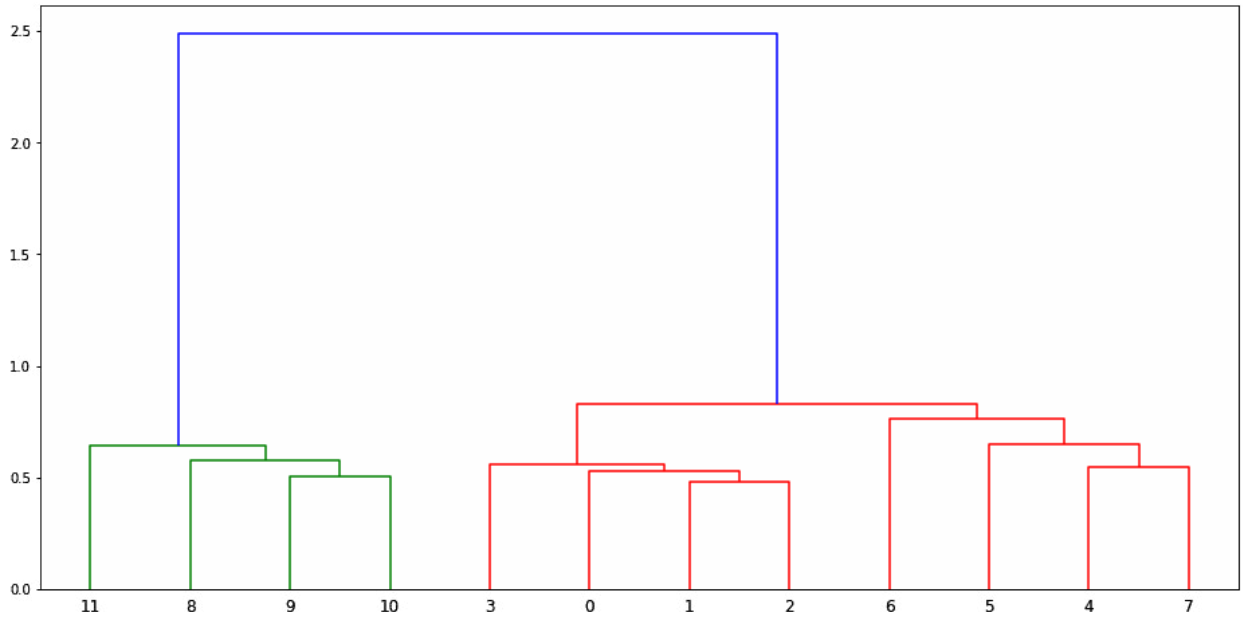

Figure 1: Dendrogram considering agglomerative clustering algorithm from growth, biochemical and phytochemical parameters data in leaves of *Passiflora alata* Curtis seedlings grown on substrates with (0-3= S1; 4-7= S2) or without (8-10= S3) coconut coir dust addition. S1= Sand + commercial substrate for seedlings (CSS) + coconut coir dust (1:1:1); S2= Sand + CSS + coconut coir dust (1.5:1.5:1); S3= Sand + CSS (1:1).

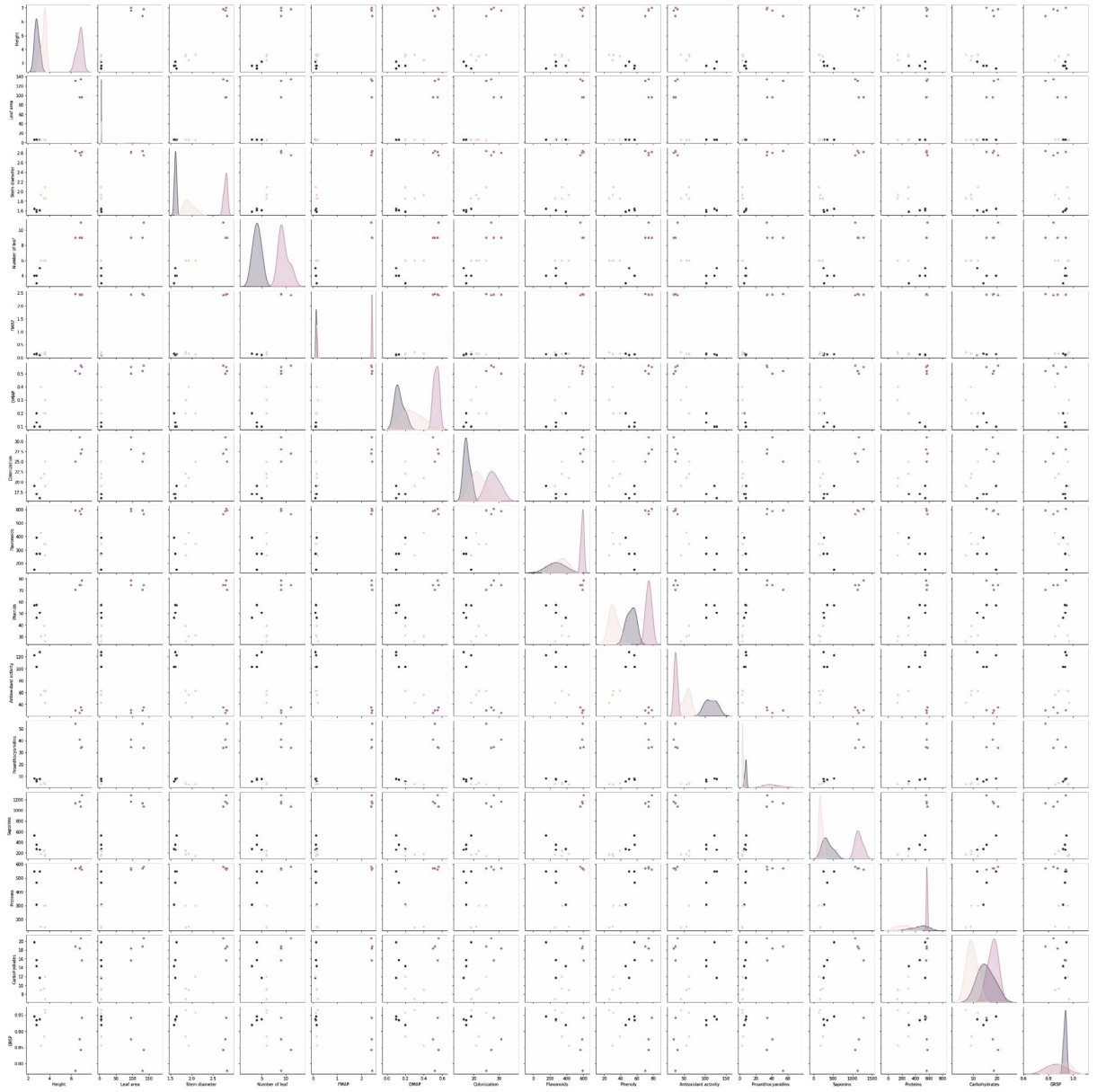

Figure 2: Clustering distribution of data for K= 3, considering the data from growth, biochemical and phytochemical parameters in leaves of *Passiflora alata* Curtis seedlings grown on substrates with or without coconut coir dust addition. 0= S1 (Sand + Commercial substrate for seedlings - CSS + coconut coir dust) (1:1:1); 1= S2 (Sand + CSS + coconut coir dust) (1.5:1.5:1); 2= S3 (Sand + CSS) (1:1).

Therefore, the augmentation of antioxidant activity occurred in plants cultivated in S2 (45.27%) and S3 (70.54%), in comparison to the seedlings cultivated in S1, considering that a lower quantity of DPPH remaining in the solution was reported to the S3 and S2 treatments (Table 2). The presence of coconut coir dust, in the highest proportion (S1), could be one of the reasons plants presented lower antioxidant activity (Table 2), similar to the results of Machado et al. (2021). Stressed plants can increase the production of reactive oxygen species (Hasanuzzaman et al., 2020), leading to the neutralization of antioxidant compounds.

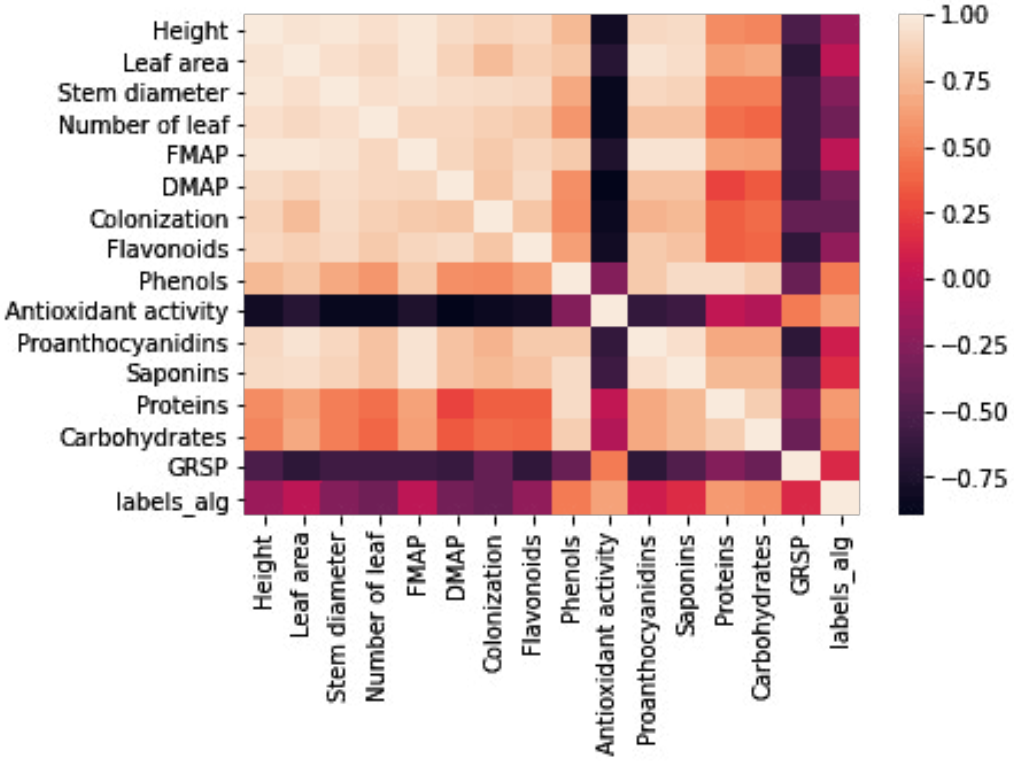

Figure 3: Correlation between the variables of growth and the biochemical and phytochemical parameters of leaves of *Passiflora alata* Curtis, cultivated in substrates with different proportions of coconut coir dust, for 68 days, in a greenhouse. DMAP= dry matter of the aerial part; GRSP= glomalin-related soil proteins; FMAP= fresh matter of the aerial part.

On the other hand, for the increment in the concentration of primary compounds, the substrate with an intermediate proportion of coconut coir dust (S2) is not recommended since the greatest biosynthesis of these molecules was obtained in seedlings cultivated with 1/3 of coir dust (S1) or without this organic substrate (S3) (Table 2). Furthermore, these primary metabolites showed a correlation with the production of secondary compounds, which were increased in plants grown on S3 (Figure 3 and Table 2).

In our study, the concentration of flavonoids in leaves of *P. alata* (587.58 mg g plant^{-1}) was higher than the one reported by other research projects with vermicompost supply (44.55 mg plant^{-1}) (Oliveira; Campops; Silva, 2015) and with coconut coir dust to *P. edulis* cultivation (415.78 mg plant^{-1}) (Oliveira et al., 2020). These results are relevant since this species' leaves are used to formulate anxiolytic herbal medicines (Fonseca et al., 2020). Thus, considering that a packet of Passiflorine® (20 tablets) has 25 mg of flavonoids expressed in vitexin, using 1 Kg of *P. alata* grown in seedlings substrates (without coir dust) is possible to produce 733.750 packets, 396.250 packets more than would be obtained from 1 Kg of *P. alata* grown in the substrate with highest of coir dust (S1). Moreover, it is possible to reduce the cost of coconut coir dust supply by about 0.10 USD, per seedling, for every 100 mL^{-1} supplied (Oliveira et al., 2020), considering that only the use of CSS mixed with sand (S3) is sufficient to obtain high flavonoid contents (Table 2).

In summary, this research shows that only the use of S3 (CSS + Sand, 1:1) is ideal to improve the growth, the total antioxidant capacity of the plant extracts, and foliar metabolite concentrations, dismissing the supply of coir dust and being superior to other studies reported for *P. alata*. Therefore, it is essential to conduct toxicity tests due to the high concentration of compounds in the extracts to enable their use in formulating anxiolytic herbal medicines based on passion fruit.

CONCLUSIONS

The use of commercial substrate for seedlings mixed with sand (1:1) (S3) is sufficient to enhance the growth, metabolite content, and antioxidant capacity of the foliar extracts of *P. alata* dispensing coconut coir dust supplementation in the growing media. Moreover, the addition of coir dust in the commercial substrate for seedlings is not recommended to optimize the production of secondary metabolites and the growth of *P. alata* seedlings.

AUTHOR CONTRIBUTION

Conceptual Idea: Muniz, B.C., Falcão, E.L. and Silva, F.S.B.; Methodology design: Muniz, B.C. and Silva, F.S.B.; Data collection: Muniz, B.C., Falcão, E.L.; Data analysis and interpretation: Muniz, B.C., Falcão, E.L., Bastos Filho, C.J.A., Silva, F.S.B.; Writing and editing: Muniz, B.C., Falcão, E.L., Bastos Filho, C.J.A., Silva, F.S.B.

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