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Management perspectives aimed at maximizing the production of secondary metabolites from medicinal plants in agroforestry systems

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Abstract Cultivation in agroforestry system represents an alternative for agroecological production for plants with economic potential, such as medicinal plants. The productive efficiency of this system requires adequate and efficient management strategies for the different intercropped species. The present study aimed to evaluate shoot biomass and production of secondary metabolites (essential oil and flavonoids) in different harvest periods (6, 12 and 18 months) from three medicinal species of economic interest, *Mikania laevigata, Varronia curassavica*, and *Fridericia chica*, organically fertilized (organic compost and vermicompost) and grown in agroforestry system with different plant density (spacing). The association of medicinal species with arbuscular mycorrhizal fungi was also evaluated and the chemical soil properties were analyzed before and after fertilization. The experiment was conducted according to the randomized block design, with four blocks and plots subdivided over time, considering the three harvest periods (6, 12 and 18 months). The cultivation of *M. laevigata, V. curassavica* and *F. chica* intercropped with *Hevea brasiliensis* and *Theobroma cacao* produced enough leaf biomass for the productive viability of medicinal plants in agroforestry systems. Fertilization with vermicompost is the most indicated for dry shoot biomass production, while for higher content of essential oil and flavonoids in the leaves of medicinal plants, the use of organic compost is more indicated. Regarding the harvest period, *Varronia curassavica* showed the highest dry shoot biomass and essential oil yield at 18 months, while for *Mikania laevigata* and *Fridericia chica*, highest production occurred at 12 months. The agroforestry system with lower density of cocoa trees enabled greater dry biomass production from the three medicinal plants and higher yield of essential oil from *M. laevigata* and *V. curassavica*. In order to obtain higher contents of flavonoids in *F. chica*, cultivation in agroforestry system with higher density of cocoa trees is recommended. The cultivation of these three medicinal plants in AFS is a viable alternative for the production of secondary metabolites of pharmaceutical interest.

Keywords *Mikania laevigata* · *Varronia curassavica* · *Fridericia chica* · Flavonoids · Essential

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oil content · Bioeconomy · Spacing · Organic fertilization

Introduction

Due to the growing global environmental concern with natural ecosystems and the consequent need for the implementation of sustainable agricultural systems, there has been an increase in the search for alternative production based on agroecology (Perfecto et al., 2010; Santos et al., 2019; Wezel et al., 2014; Trabelsi et al., 2019), such as agroforestry systems. (AFS) (Brunetti et al., 2013; Czelusniak et al., 2012; Farias, 2018). For the efficient functioning of these systems with a view to gains in productivity and rational land use, it is necessary to consider the choice of plant species that will compose the system, management strategies and socioeconomic profile of farmers (Cardoso et al., 2017; Montagnini, 2017).

Regarding the use of species, the cultivation of medicinal species in agroforestry systems may associate production diversification with increase in income for rural producers (Bari and Rahim, 2012; Paulus et al., 2019), in addition, the use of these species in AFS is also an important instrument for the conservation of these plant genetic resources (Chirwa et al., 2008; Amujoyegbe et al., 2012; Dassou et al., 2019). Among tropical countries, Brazil has exuberant diversity of native flora, with potential for use in the pharmaceutical industry, especially medicinal plants (Valli et al., 2018). In recent years, the sharp increase of the use of these plants in the production of herbal medicines from secondary metabolites has received great motivation from government health programs, which have recommended their use and encouraged sustainable agricultural production (Ribeiro, 2019). Despite the economic potential of these plants, they are not cultivated, they are harvested using the extractivist method. There are few studies addressing the adequate agronomic management for the cultivation of these plants (Vásquez et al., 2014), especially in AFS.

The increased demand for secondary metabolites from medicinal plants requires the establishment of cultivation strategies in sustainable production systems that guarantee quality raw material and regular supply of these plant genetic resources (Singh and Sharma, 2020). Adequate management perspectives can be based on several aspects, such as fertilization, shading and harvest period. As agro-ecological systems aim at reducing the use of chemical inputs, organic fertilization is a suitable alternative for sustainable use to contribute to improve soil quality properties, such as its chemical characteristics (Pessoa et al., 2015; Rao et al., 2004), in addition to presenting the possibility of reducing production costs (Case and Jensen, 2019). Still in relation to soil quality aspects, it is noteworthy that the absorption of certain nutrients from the soil may be associated with the capacity of species to associate with arbuscular mycorrhizal fungi (Matsubara et al. 2008, Zubek et al. 2015), leading to increased productivity (increased biomass) (Souza et al., 2006) and production of primary metabolites, which are precursors of secondary metabolites (Manoharan et al. 2010). As fertilization, it is also important to consider the shading condition provided by the density of plants of other species present in the cultivation system. This factor can influence plant growth and the production of secondary metabolites from medicinal plants (Barros et al. 2009; Botrel et al., 2010). It is also important to pay attention to the harvest period, which has relevance in estimating economic return for the rural producer as a function of time (Wu et al., 2020).

Species *Fridericia chica* (Bonpl.) L.G. Lohmann, *Varronia curassavica* Jacq and *Mikania laevigata* Schultz Bip. Ex Baker are medicinal plants native to Brazil that have secondary metabolites with recognized economic and pharmaceutical potential, and therapeutic properties related to their anti-inflammatory, astringent, bronchodilator, anti-allergic and anti-asthmatic characteristics (Brunetti et al., 2013; Czelusniak et al., 2012; Farias, 2018). It should be noted that the main secondary metabolite with use value for *F. chica* are flavonoids, while for *V. curassavica* and *M. laevigata* are essential oils. Considering that the hypothesis is that vermicompost and organic compost, as they are complex fertilizers (which contain, in addition to macro and micronutrients, organic compounds that stimulate plant growth) can increase the growth and production of secondary metabolites of cultivated medicinal plants in agroforestry systems, stimulating mycorrhization, this study aimed to evaluate the harvest period and use of organic fertilizers in the cultivation of these three species under different shade conditions (density of *Theobroma cacao* plants) in AFS in the southern region of Bahia, Brazil, aiming at increasing biomass and essential oil production.

Material and methods

Description of the experimental area

The experiment was conducted in an experimental AFS area of the Cocoa Research Center—CEPEC, belonging to the Executive Committee of the Cocoa Crop Plan—CEPLAC—Ilhéus (Bahia, Brazil), from March 2016 to September 2017. Climatic data obtained during the experiment showed average monthly temperature of 23.7 °C with average monthly rainfall of approximately 118.4 mm. The AFS demonstrative unit was installed in December 1999, and consists of intercropping between *Theobroma cacao* and *Hevea brasiliensis* plants. The area has 4500 m^2 and consists of rubber trees of the SIAL 893 clone arranged in double rows spacing 15 m between rows and 3 × 2.5 m inside the row and by cacao trees of Theobahia variety spacing 3 × 3 m (Marques et al., 2004). Seedlings of *M. laevigata, V. curassavica* and *F. chica* medicinal species were planted inside the AFS. Seedlings were produced by cutting and hardened in greenhouse for three months before planting in the field. At the time of planting of medicinal plants, cocoa trees had, on average, one meter in height, which were submitted to annual pruning, and rubber trees were 20 m in height.

Experimental design

The experiment was carried out in a randomized block design, with split plots over time, considering factor fertilization with different types of fertilization, without fertilization (CC), organic compost (CP) and vermicompost (VR), conditioned to harvest periods of 6, 12 and 18 months. Thus, nine treatments were divided into four blocks. The main source of organic compost was sheep manure, restaurant waste and green grass straw, with C/N ratio of 8.35. Regarding vermicompost, it is a biological process carried out by earthworms, which main source of this fertilizer was cattle manure, with C/N ratio of 8.37. The use of blocks was established to minimize the heterogeneous effect of the environment in the experimental area. This design was proposed for each medicinal plant species (*M. laevigata*, *V. curassavica*, and *F. chica*). The planting of the three medicinal species was carried out in March 2016, spaced 1.5 m between plants and 4 m between blocks, totaling 18 plants per species in each block (Appendix A), with six plants for each treatment. Fertilization at planting was performed, and every three months, top dressing was performed, applying 1 kg of fertilizer for each treatment.

Soil analysis

At the beginning of the experiment, soil samples were collected from the experimental area using 40 simple zigzag samples throughout the area at depth from 0 to 20 cm, forming a composite sample for each block and sent to the Laboratory of Soil, Plant Tissue and Fertilizer Analysis of the Federal University of Viçosa, Viçosa, state of Minas Gerais, Brazil and Laboratory of Soil Analysis, Plant Tissue and Fertilizer—Federal University of Viçosa for analysis (see Appendix B). The soil chemical characterization was performed according to EMBRAPA methodology (Donagema et al., 2011). Soil pH was determined at soil: water ratio of 1:2.5 (m/m). Magnesium (Mg), calcium (Ca), aluminum (Al) were extracted with 1 M KCl and quantified through atomic absorption spectrometry (for Ca and Mg) and titration with 0.025 M NaOH (for Al). Potassium (K) and phosphorus (P) were extracted using Mehlich 1 method and quantified through flame spectrometry (K) and blue-Mo method (P). Soil organic matter (OM) was determined using the Walkley and Black procedure. Micronutrients copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) were extracted by Mehlich 1 and quantified by atomic absorption spectrometry. Cation exchange capacity (CEC) was calculated as the sum of exchangeable bases and exchangeable acidity.

Variables analyzed

Biomass and essential oil content

The dry shoot biomass of each plant was evaluated every six months and essential oil extraction was performed in triplicate from 100 g of leaf. Leaves were dried in forced ventilation oven at 40° C and then submitted to hydrodistillation with Clevenger apparatus. Leaves were immersed in 1500 ml of distilled water. The extraction time was different for each species, for *M. laevigata* of 240 min and for *V. curassavica* of 90 min. After extraction, the essential oil was added of anhydrous sodium sulfate to remove residual water, thus obtaining the essential oil, which was stored in amber glass bottles. The essential oil content expressed as percentage (%) was obtained from the relationship between mass of extracted oil (g) and dry leaf mass in the flask (g), multiplied by 100. The essential oil yield (g $plant^{-1}$) was estimated from the relationship between oil content (%) and dry leaf mass per plant.

Total flavonoid content

After the determination of semiannual biomass, carried out in the determination of total flanonoids, *F. chica* leaves were dried at temperature of 40° C to constant mass, ground in knife mill with 4.0 mm sieves and stored in black polyethylene bags using methodology of Peixoto et al., (2008) with adaptations. About 0.600 g of sample were weighed and transferred to 50.0 mL Erlenmeyer flasks with 20.0 mL of methanol and heated and stirred under gentle boiling in heating plate for 30 min. Extracts were filtered on filter paper and quantitatively transferred to 25.0 mL volumetric flasks and the volume was measured with methanol. The solution was clarified with 3 ml of Ba $(OH)_2$ and 3 ml of $ZnSO_4$. An aliquot of 2000 µL was removed from the sample solution into a 10.0 mL volumetric flask, with 60 µL of glacial acetic acid, 1000 µL of 20% methanolic pyridine solution and 250 µL of aluminum chloride, with volume being checked with distilled water. After 30 min, spectrophotometric reading was performed at 420 nm in triplicate. For the quantification of flavonoids, analytical curve was prepared with methanolic solution of Rutin standard, obtaining concentrations of 5; 10; 15; 20 and 25 µg.mL^{-1}, prepared from the stock solution of 5.0 mg mL^{-1}. Aliquots of 100; 200; 300; 400 and 500 µL of each concentration obtained were submitted to the same procedure performed with plant samples, and absorption readings were performed at 420 nm in triplicate.

Arbuscular mycorrhizal fungi

To evaluate colonization of arbuscular mycorrhizal fungi, the finest roots of each medicinal species were selected and collected, which were washed in sieve with tap water to remove all soil particles and stored in plastic containers containing 70% ethanol for preservation. In the laboratory, roots were clarified and stained according to the modified Phillips and Hayman method (1970). Roots were transferred to an identified test tube containing 10% KOH solution. Tubes were heated in water bath for 20 min and roots were washed under running water to remove excess KOH. Tubes were added of 10% hydrogen peroxide solution for two hours, and then, roots were washed in distilled water. After washing, roots were acidified using 5% aqueous HCl solution for 5 min. For root staining, fountain pen ink solution (5%) was added to the tube and left in water bath (60 °C) for 1 min. Stained roots were observed under stereoscopic microscope to assess colonization percentage. Colonization quantification was performed using the intersection method (McGonigle et al., 1990). The colonization percentage of segments was based on the relationship between the number of intersections of colonized roots and the total number of intersections of segments observed, the result being multiplied by 100.

Statistical analysis

To verify the effect of types of fertilizers and different harvest periods, the residues of models were initially extracted and submitted to the Shapiro–Wilk normality test and Bartlett's homoscedasticity test. Since the aforementioned assumptions have been met, data were submitted to analysis of variance and subsequently, the average values of the dry shoot biomass (DSB), essential oil content (OC), essential oil yield (OY), total flavonoids (TF) and colonization of mycorrhizal fungi (CAMF) were compared using Tukey's test at 5% probability.

Principal component analysis (PCA) was carried out in order to verify the behavior of the combination of each species with types of fertilizer, arranged in different conditions of experimental blocks (plant density). For this analysis, chemical soil analysis, average biomass data and colonization of mycorrhizal fungi variables were considered. The number of PCs was chosen, considering only components with eigenvalue greater than one. The biplot chart was used to represent the first two principal components. In addition, graphs showing the weight and the significant contribution of variables were produced. This significance is related to the eigenvector greater than 0.7 (Zwick and Velicer, 1982). All statistical analyses were performed using the R software version 3.6.2 (R Desenvolvimento Core Team R, 2021).

Results

The medicinal plant species under study, *M. laevigata, V. curassavica*, and *F. chica* submitted to two different types of fertilization responded differently in three harvest periods with respect to the essential oil content (OC) and total flavonoids (TF), oil yield (OY), dry shoot biomass production (DSB) and colonization of arbuscular mycorrhizal fungi (CAMF). For this reason, results are shown and discussed separately for each of the three medicinal plant species.

For species *M. laevigata, d*ry shoot biomass (DSB) was influenced by the harvest period and type of organic fertilizer applied. Harvest performed at 18 months after the beginning of the experiment resulted in 0.59 kg per plant and yield of 653.1 kg ha^{-1}. In relation to fertilizer, vermicompost promoted greater DSB production, with 0.59 kg per plant and yield of 653.1 kg ha^{-1}. Regarding colonization of arbuscular mycorrhizal fungi (CAMF), species showed effect of fertilization and harvest time independently. The highest CAMF was at 18 months, with 59.3% of colonized roots, while the different types of fertilizers did not influence colonization (Table 1). Regarding oil content (OC) and oil yield (OY), there was interaction between harvest period and type of fertilizer. For OC, the highest content occurred without the use of fertilizer and showed no statistical difference at 12 and 18 months, while for OY, the highest average value at 18 months, without the use of fertilization (Table 2).

For species *V. curassavica*, dry shoot biomass (DSB) showed no differences between harvest periods of 12 and 18 months, and the average production for both harvest periods was 0.80 kg per plant, with yield of 896.8 kg ha $^{-1}$. Regarding oil content (OC) and oil yield (OY), fertilization and harvest period influenced these variables independently. The species presented the highest OC (0.37%) and OY (13.5 g/plant) at 12 months. The compost fertilizer also provided the best OC (0.27%) and OY (9.89 g / plant) results, when compared to treatment without fertilizer (Table 3). Colonization of arbuscular mycorrhizal fungi (CAMF) was influenced by the interaction between harvest periods and types of fertilizers. The highest CAMF rate (69.0%) occurred at 18 months in plants fertilized with vermicompost (Table 4).

For species F. chica, dry shoot biomass (DSB), total flavonoids (TF), and colonization of arbuscular mycorrhizal fungi (CAMF) showed effect of fertilization and harvest period independently. The highest DSB production occurred in the 12-month harvest period, resulting in production of 0.17 kg plant^{-1} and yield of

Table 1 Average values for dry shoot biomass (DSB) and colonization by arbuscular mycorrhizal fungi (CAMF) of *M. laevigata* grown in agroforestry system submitted to organic fertilization and harvested at 6, 12 and 18 months after the beginning of the experiment

Variables			DSB (g)	CAMF (%)
M. laevigata	Harvests (months)	6	476.2(± 123.3)b	46.2(± 7,5)b
		12	929.1(± 276.9)b	46.3(± 7.5)b
		18	2351.4(± 834.0)a	59.3(± 11.8)a
	Fertilizers	CP	1587.5(± 682.2)b	53.0(± 12.2)a
		VR	2351.4(± 942.2)a	57.2 (± 5.91)a
		CC	1364.9(± 658.0)b	59.3 (± 10.53)a

Legend: *CP*, organic compound; *VR*, vermicompost; *CC*, no fertilizer

* Means followed by the same letter in column do not differ statistically from each other using the Tukey test p ≤ 0.05

Table 2 Average values for content (OC) and yield (OY) of essential oil from *M. laevigata* grown in agroforestry system submitted to organic fertilization and harvested at 6, 12 and 18 months after the beginning of the experiment

Variables	Fertilizers	Harvests (months)		
		6	12	18
OC (%)	CP	0.14(± 0.01)ABc	0.13(± 0.005)Bc	0.15(± 0.01)Ac
	VR	0.16(± 0.02)Bb	0.17(± 0.02)Bb	0.20(± 0.01)Ab
	CC	0.19(± 0.01)Ba	0.22(± 0.01)Aa	0.24(± 0.01)Aa
OY (g plant^{-1})	CP	0.430(± 0.2)Ba	0.842(± 0.4)Aba	2.105(± 1.3)Ab
	VR	0.577(± 0.2)Ba	1.220(± 0.5)Ba	3.185(± 1.9)Ab
	CC	0.900(± 0.1)Ba	2.087(± 0.5)Ba	5.637(± 1.1)Aa

Legend: *CP*, organic compound; *VR*, vermicompost; *CC*, no fertilizer

* Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ from each other by the Tukey Test $p < 0.05$. Legend: OC (Oil content) and OY (Oil yield)

Table 3 Average values for dry shoot biomass (DSB), content (OC) and yield (OY) of essential oil from *V. curassavica* grown in agroforestry system submitted to organic fertilization and harvested at 6, 12 and 18 months after the beginning of the experiment

Variables			DSB (g)	OC (%)	OY (g plant^{-1})
V. curassavica	Harvests (months)	6	732.2(± 243.9)b	0.20(± 0.04)b	1.55(± 0.7)c
		12	3683.1(± 831.0)a	0.37(± 0.1)a	13.5(± 5.4)a
		18	2775.1(± 835.1)a	0.23(± 0.04)b	6.75(± 2.89)b
	Fertilizers	CP	2947.5(± 1597,3)a	0.27(± 0.09)a	9.89(± 7.41)a
		VR	2352.7(± 1344.6)a	0.23(± 0.09)ab	6.36(± 5.69)ab
		CC	1890.2(± 1208.0)b	0.20(± 0.07)b	4.02(± 4.0)b

Legend: *CP*, organic compound; *VR*, vermicompost; *CC*, no fertilizer

* Means followed by the same letter in the column do not differ statistically from each other using the Tukey test $p \leq 0.05$. Legend: DSB (dry shoot biomass), OC (oil content) and OY (oil yield)

Table 4 Average values for colonization of arbuscular mycorrhizal fungi (CAMF) of *V. curassavica* grown in agroforestry system submitted to organic fertilization and harvested at 6, 12 and 18 months after the beginning of the experiment

Variable	Fertilizers	Harvests (months)		
		6	12	18
CAMF (%)	CP	64.6(± 10.3)Aa	61.8(± 10.3) Aa	52.9(± 6.1) Ab
	VR	52.2(± 5.3)Ba	52.3(± 5.3) Ba	69.0(± 10.8) Aa
	CC	53.2(± 3.6) Aa	53.2 (± 7.7)Aa	57.9(± 19.6) Aab

Legend: *CP*, organic compound; *VR*, vermicompost; *CC*, no fertilizer

* Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ from each other by the Tukey Test $p < 0.05$

Table 5 Average values for dry shoot biomass (DSB), total flavonoid (TF), and colonization by arbuscular mycorrhizal fungi (CAMF) of *F. chica* grown in agroforestry system submitted to organic fertilization and harvested at 6, 12 and 18 months after the beginning of the experiment

Variables			DSB (g)	TF µg/100 mg	CAMF (%)
F. chica	Harvests (months)	6	421.5(± 91.6)b	0.13(± 0.02)b	45.7(± 6.4) b
		12	668.8(± 112.7)a	0.23(± 0.1)a	45.8(± 6.4)b
		18	396.9(± 92.4)b	0.19(± 0.07)ab	62.2(± 15.3)a
	Fertilizers	CP	290.3(± 159.2)b	0.17(± 0.08)a	47.7(± 12.9) a
		VR	396.9(± 140.0)a	0.18(± 0.07)a	49.1(± 13.6)a
		CC	249.9(± 149.4)b	0.17(± 0.06)a	40.3(± 8.2)a

Legend: *CP*, organic compound; *VR*, vermicompost; *CC*, no fertilizer

[*] Means followed by the same letter in the column do not differ statistically from each other using the Tukey test p ≤ 0.05

185.6 kg ha^{-1}. In relation to fertilizer, vermicompost promoted higher DSB production values, with 0.09 kg per plant and yield of 110.2 kg ha^{-1}. In relation to TF, the highest levels occurred at 12 and 18 months, with the highest content at 12 months with 0.23 µg per 100 mg of leaf, respectively, while type of fertilizer did not influence TF (Table 5). For CAMF, species showed greater colonization at 18 months, with 62.2% of colonized roots, and type of fertilizers did not influence CAMF (Table 5).

Principal component analysis (PCA) revealed four significant major components (PC1, PC2, PC3 and PC4) to explain variations between treatments (Appendix B). The cumulative variance of components was 80.8%. Regarding the contributions of variables within each component, it was observed that PC1 explains the greatest variation (50%) between treatments and V, SB, Ca, CEC, P-Rem, Mg, MO, K, P, H + Al and pH variables are significant for this component, which has V (base saturation) and pH with the highest and lowest contribution weight, respectively. PC2 explained 12% of data variation between treatments and DSB, Fe, H + Al, pH, CEC, AMF and Mg variables proved to be significant. While PC3 explained 10% of data variation, significant variables were the Zn, Mn, P and Cu. PC4 explained only 8% of data variation and significant variables were CAMF, Cu and DSB (Appendix C). PCA was able to separate species grown with different types of fertilization depending on the difference in plant density in the experimental area.

PCA grouped *V. curassavica* (VC) in the system with lower plant density combined with fertilization with organic compost (CP) and vermicompost (VR), as well as *M. laevigata* (ML) combined with fertilization with vermicompost (VR), and *F. chica* (FC) without fertilization (CC) with association with variables Ca, CAMF, DSB, CEC, Mg, OM, SB and V. However, the same association occurs for *M. laevigata* (ML) without fertilization (CC) in the system with higher density. *F. chica* (FC) combined with fertilization with vermicompost (VR) and without fertilization (CC) and *M. laevigata* (ML) combined with organic compost (CC) and without fertilization (CC) were associated with variables P, K, Cu, P-Rem and pH (Fig. 3). Regarding the angle formed between vectors of variables, the correlation between DSB and CAMF stands out. In addition, the analysis grouped species cultivated in the system with the highest density, *M. laevigata* (ML) and *V. curassavica* (VC) combined with all fertilization levels associated with variables H + Al, Fe and Mn. The same occurs for *V. curassavica* (VC) without fertilization (N) in the system with the highest density (Fig. 1).

Discussion

The guarantee of meeting the demand for commercialization of secondary metabolites from different medicinal plants requires specific management practices and adequate cultivation system for the full development of agricultural production (Atawodi et al., 2017; Tanga et al. 2018). Our study presents management perspectives for three medicinal plant species native to Brazil with high economic value and

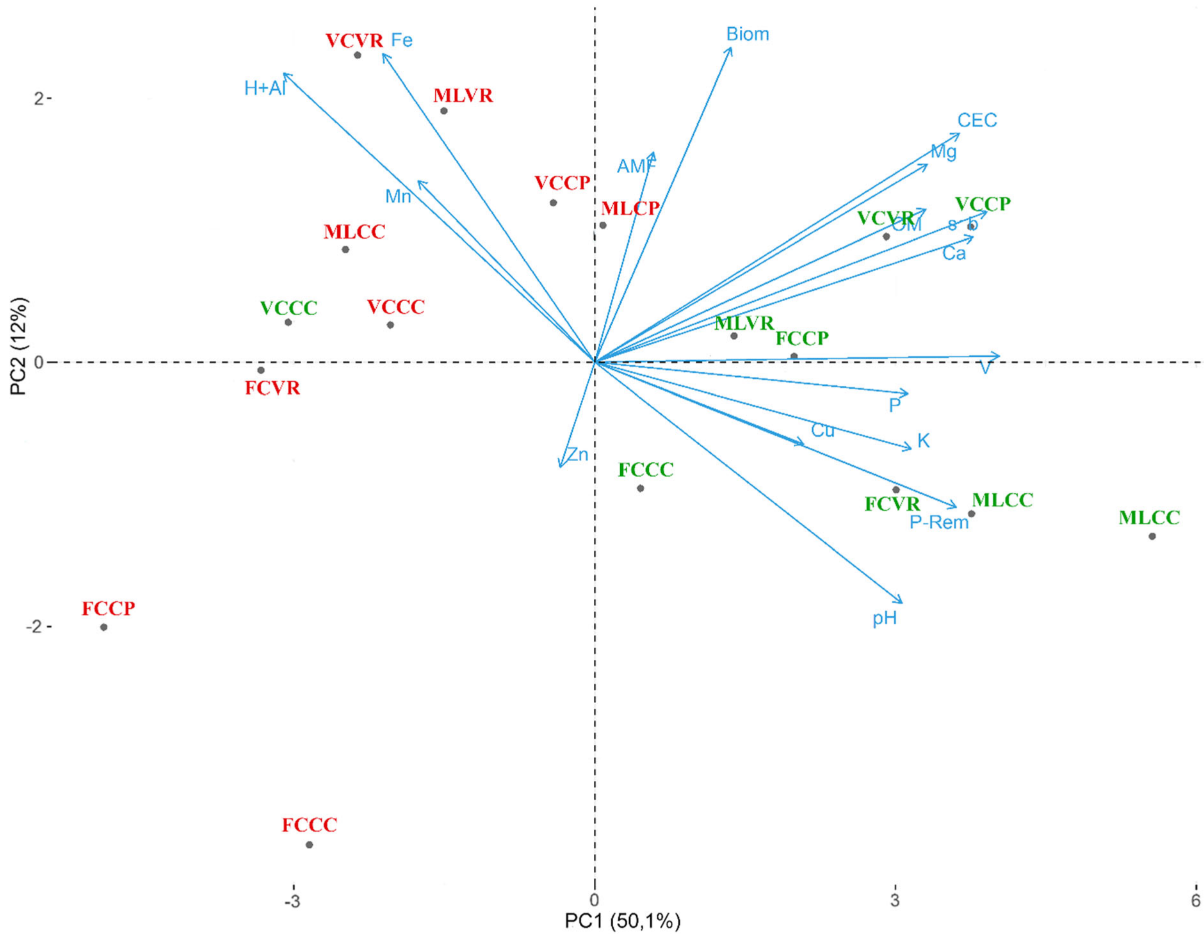

Fig. 1 Biplot chart with medicinal plant species (VC: *Varonia curassavica*; ML: *Mikania laevigata*; FC: *Fridericia chica*) and types of fertilization (C: Compound; V: Vermicompost; N: Without fertilization;) for variables: V (base saturation), Ca (calcium content), CEC (cation exchange capacity), pH H_2O (pH in water), P-Rem (remaining phosphorus content), Mg (magnesium content), OM (soil organic matter), K (potassium content), P (phosphorus content), H + Al (potential acidity), SB (sum of bases), Zn (zinc content), Fe (iron content), Mn (manganese content), Cu (copper content), CAMF (arbuscular mycorrhizal fungi) and Biom (Biomass). Green coloring indicates plots located in lower density of cocoa plants while red coloring indicates plots located in higher density of cocoa plants

pharmaceutical interest. According to our findings, these species have different behavior in relation to experimental conditions and respond positively when inserted in an agroforestry system.

The differentiated expression of dry shoot biomass production (DSB) is directly related to the biological specificities of each species. Therefore, the production of species V. curassavica should be highlighted, which has the possibility of annual harvesting, with the use of fertilizer, which confirms the already recognized productive potential of the species (Capaz, 2017). Considering that the expression does not depend only on the intraspecific factor, but also on abiotic factors, the use of vermicompost to increase *F. chica* and *M. laevigata* biomass stands out. Since it has high concentration of macronutrients and works as great microbial population growth promoter, which in turn participates in the bioavailability of these mineral nutrients, this fertilizer satisfactorily influences plant growth (Amaral et al., 2017; Schumacher et al., 2001). In this sense, vermicompost is promising for the reality conditions of rural producers in the study region, to the detriment of high availability and economic return of flavonoids and essential oils, strengthening the development strategies of the productive chain of this species.

In general, essential oil content and yield, as well as total flavonoids were positively influenced by the organic compound, which provides high levels of nutrients (Chagas et al., 2011; Sales et al., 2009). The lack of *M. laevigata* response regarding this type of fertilization for the production of secondary metabolites (essential oil) can be explained by the low requirement for nutrients (Pereira et al., 1998), which were provided by the agroforestry cultivation system itself, which has large amounts of accumulated organic matter (4% organic matter content in the soil). Due to litter deposition, biodiversity and high nutrient cycling, AFS have good distribution of nutrients along the soil profile caused by species stratification and differentiated rooting (Iwata et al. 2012).

In addition to the assertive fertilization choice, the optimum harvest period was also relevant, which will ensure the best agricultural management of the three medicinal plant species. The greatest interference in the content and yield of secondary metabolites was observed in the harvest periods, where there is influence of seasonality and temperatures (Gobbo-Neto and Lopes, 2007). For *V. curassavica* and *F. chica* species, the highest contents of essential oil and flavonoids occurred in the hot season, from September 2016 to March 2017, whereas, for *M. laevigata*, the highest levels occurred in colder seasons, autumn and winter. The season of the year when the plant is collected is a key factor in the production of secondary metabolites. The quantity and nature of active constituents are not constant during the year. Several classes of secondary metabolites present seasonal variations such as essential oils, sesquiterpene lactones, flavonoids, coumarins, saponins, alkaloids, tannins and phenolic acids (Gobbo-Neto and Lopes, 2007).

The combined analysis of variables (PCA) demonstrated the formation of two groups of medicinal plants according to the density of cacao trees (*Theobroma cacao*) adopted in AFS. Variables dry shoot biomass and mycorrhization by CAMF in *M. laevigata*, *V. curassavica* and *F. chica* were strongly influenced by the lower density of cocoa trees in AFS. Therefore, a lower density of *Theobroma cacao* plants in AFS can be considered a strategic factor to maximize the efficiency of the use of different resources (especially sunlight) by intercropped medicinal plants. The content of secondary metabolites in a plant species can be influenced by several biological and environmental factors (Gouvea et al., 2012). Light, both in terms of quality and quantity, is one of the most important environmental factors that affect the synthesis of these compounds (Gobbo-Neto and Lopes, 2007).

In AFS with lower cocoa density, plant development was significantly correlated with the application of organic fertilizer, which in addition to being rich in macronutrients essential to the nutrition of plants such as N, P, K, Mg and S, may contain N_2 fixing bacteria (Schumacher et al., 2001). Organic compounds are considered fertilizers with low content of readily available nutrients, containing only 10% or 20% of nutrients found in soluble synthetic fertilizers (Souza et al., 2012); however, they have wide action spectrum, also acting on the physical and biological soil properties, undergoing continuous mineralization in the medium and long terms (Silva et al., 2015). The addition of organic fertilizers to the soil; therefore, has several positive aspects, demonstrating to be a viable practice in increasing plant productivity and the sustainability of agrosystems (Souza et al., 2012).

In AFS, the nutritional efficiency of plants depends on several factors, such as climatic, edaphic, genetic (intrinsic to species), among others that affect the absorption and utilization of nutrients by plants (Smith and Smith, 2011; Iwata et al., 2012). An investigative focus on these factors shows effective results of medicinal plants grown in this type of system, with increased productivity and higher net profit per area unit compared to monocultures (Khatun et al. 2010; Sujatha et al., 2011). In this way, our research complements AFS efficiency results for the cultivation of medicinal plants based on adequate management.

Conclusions

The cultivation of medicinal plants *Mikania laevigata, Varronia curassavica* and *Fridericia chica* in agroforestry system composed of *Hevea brasiliensis* and *Theobroma cacao* is a promising alternative for the production of secondary metabolites of pharmaceutical interest.

Vermicompost organic fertilizer is the most suitable for the production of dry shoot biomass while for higher contents of essential oil and flavonoids, organic compound is more efficient.

For the harvest period, *Varronia curassavica* showed higher DSB production and OY at 18 months, while for *Mikania laevigata* and *Fridericia chica*, better results were obtained at 12 months.

Agroforestry system with lower density of cocoa trees enabled greater dry biomass production from the three medicinal plants and higher essential oil yield from *M. laevigata* and *V. curassavica*. In order to obtain higher contents of flavonoids in *F. chica*, cultivation in agroforestry system with higher density of cocoa trees is recommended.

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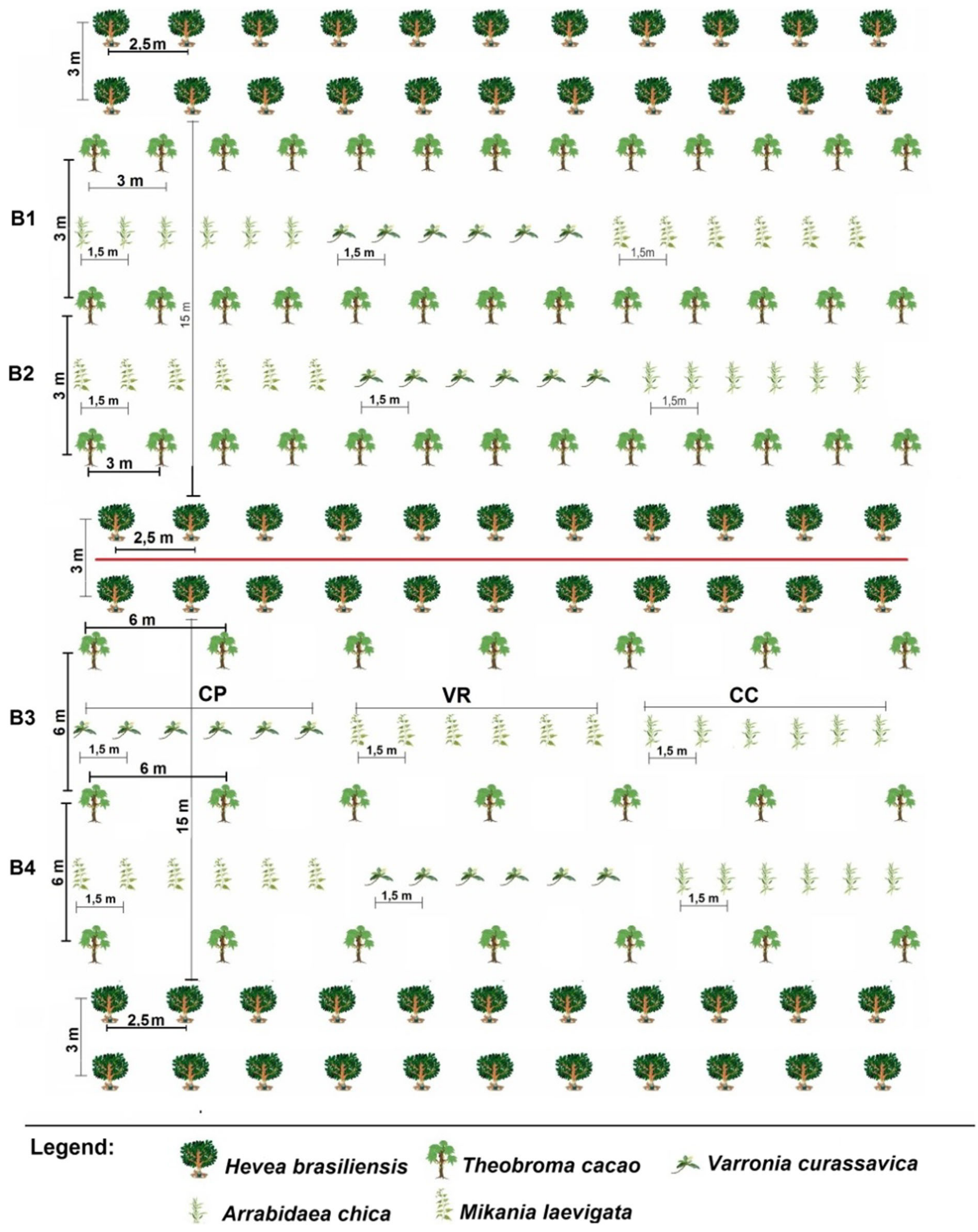
Appendix

Appendix A

Schematic drawing of the experimental area with medicinal species grown in agroforestry system, fertilized with organic compost (CP) and vermicompost (VR) and non-fertilized plants (CC), Ilhéus-BA, 2018. B1, B2, B3 and B4 represent the blocks (replicates).



3 m
2,5m
B1
3 m
3 m
1,5 m
1,5 m
1,5m
15 m
B2
3 m
1,5 m
1,5 m
1,5m
3 m
3 m
2,5 m
6 m
B3
6 m
CP
VR
CC
1,5 m
1,5 m
1,5 m
6 m
15 m
B4
6 m
1,5 m
1,5 m
1,5 m
3 m
2.5m
Legend:
Hevea brasiliensis
Theobroma cacao
Varronia curassavica
Arrabidaea chica
Mikania laevigata

Appendix B

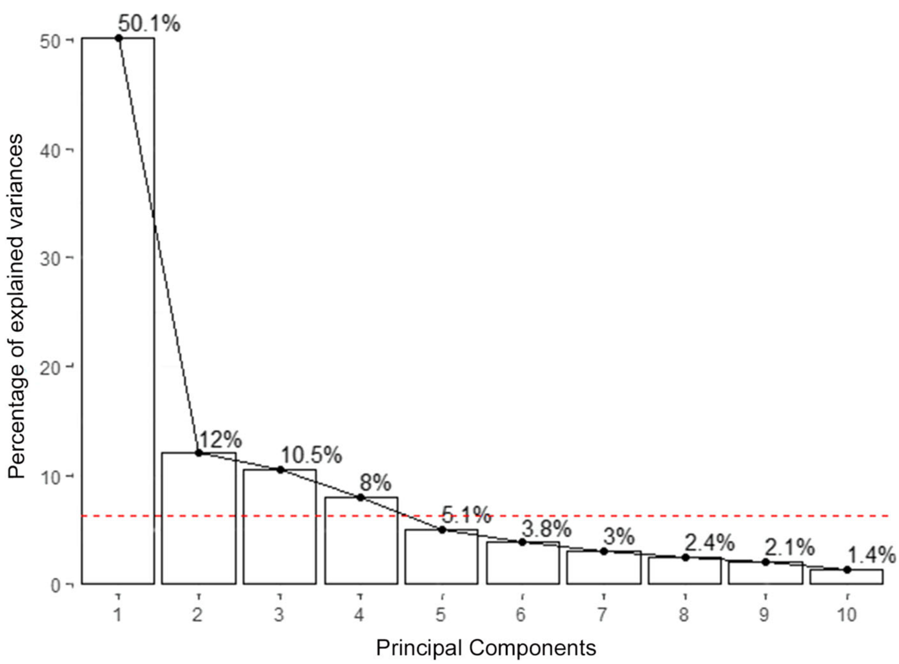
Chemical soil characteristics before experiment implantation.

Block	pH (H_2O)	c	P mg dm^{-3}	K	Ca^{2+} cmol$_c$dm^{-3}	Mg^{2+}	Al^{3+}	H + Al	SOB*	CEC	V %	P-Rem mg L^{-1}	OM %	Cu mg dm^{-3}	Mn	Fe	Zn
1	5.89		8.6	32	8.2	4.38	0	4.5	12,71	17.21	73.9	13.2	4.39	1.2	146.7	33.9	3.53
2	5.27		9.4	56	5.32	4.03	0	5.8	9,49	15.29	62.1	13.5	3.77	2.78	320.3	42.7	8.58
3	5.53		8.2	40	7.99	4.05	0	4.7	12,14	16.84	72.1	18.2	3.77	1.8	202.7	23.2	9.43
4	5.17		15.7	72	8.21	5.7	0	5.5	14,09	19.59	71.9	14.9	4.08	3.01	170.3	35	4

*SOB = sum of exchangeable bases; CEC = cation exchange capacity; V = base saturation.

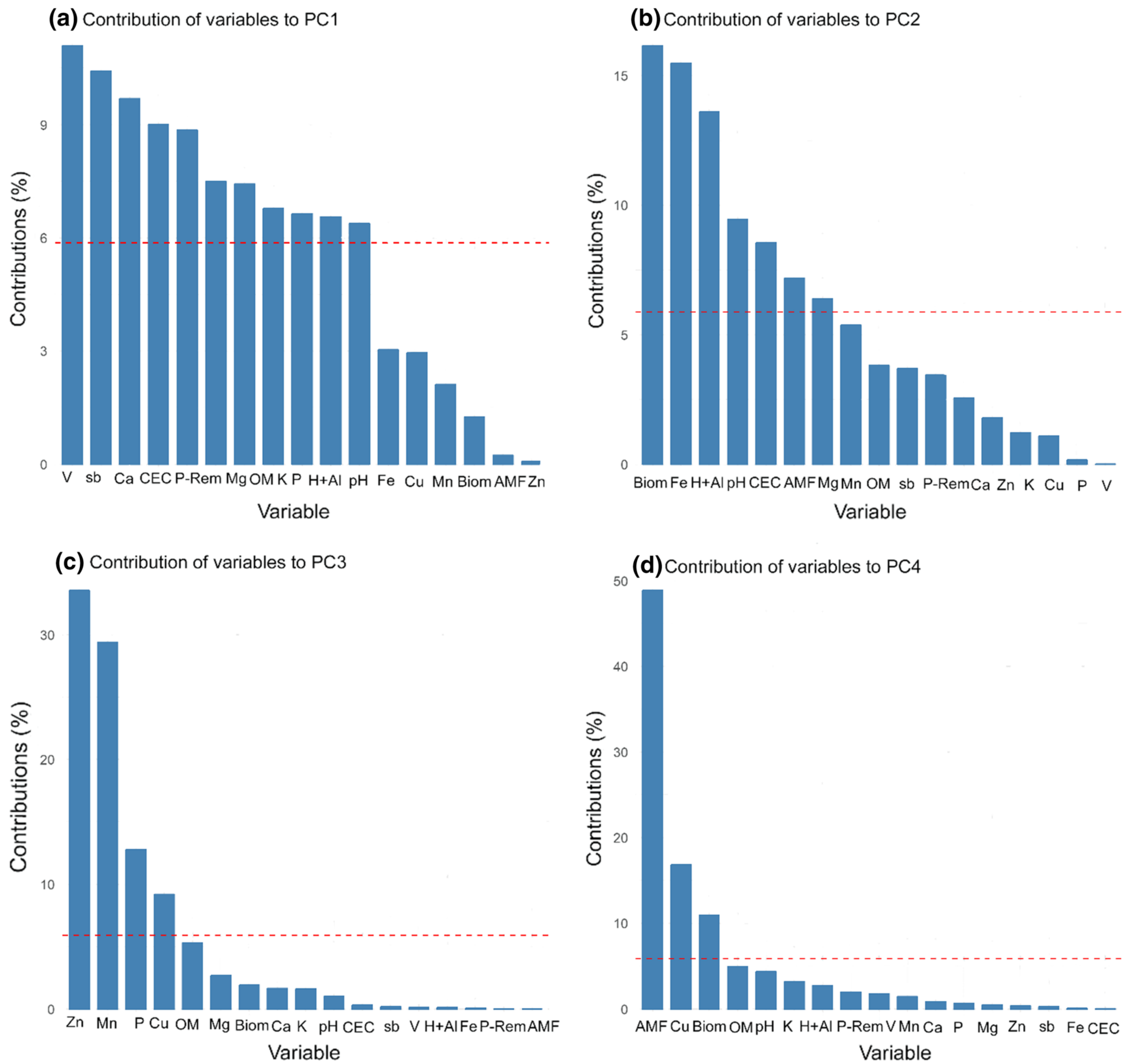
Appendix C

Principal components (PCs) obtained and their respective percentages of explained variances. Components above the red line have eigenvalue greater than one.



Appendix D

Weighting coefficient for each variable: a. First principal component (PC1); B. Second principal component (PC2); C. Third principal component (PC3); D. Fourth principal component (PC4). Variables above the red line have eigenvalue > 0.7.



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