

Agronomical and phytochemical aspects of fáfia

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ABSTRACT

The medicinal species fáfia (*Hebanthe eriantha*), is a product of extractivism in the region of the Paraíba Valley, São Paulo state, Brazil, with endangered genetic variability. This study did an agronomical characterization and an analysis of active compounds of five accessions. This research is a partnership of UNESP-Botucatu Medicinal Plants Laboratory, Paraíba Valley Center (APTA) and CPQBA-UNICAMP Agrotechnological Division. A field experiment using completely random blocks with five accessions and seven replications was used. The stem and leaf wet/dry weights, root wet/dry weight, length of the longest stem, foliar area, foam index and pfaífic acid content samples were evaluated. Accession I1800 had root dry weight, stem and leaf dry weight, and foliar area greater than other accessions (176.16 g/plant, 7.301 kg/plant, 155.04 cm²). Pfaífic acid content was similar to other accessions (0.640-0.366 %mm⁻¹) Among all five accessions, there is a positive correlation among the foliar area and stem and leaf dry weight and between the foliar area and root dry weight.

Keywords: *Hebanthe eriantha*, medicinal plants, cultivation, pfaífic acid, Amaranthaceae.

RESUMO

Aspectos fitotécnicos e fitoquímicos de acessos de fáfia

A espécie medicinal fáfia (*Hebanthe eriantha*), é um recurso extrativista da região do Vale do Paraíba, cuja variabilidade genética encontra-se ameaçada. Neste estudo realizou-se a caracterização agrônômica e análise de princípio ativo de cinco acessos. Este estudo é uma parceria entre o Laboratório de Plantas Medicinais da UNESP-Botucatu, o Pólo Vale do Paraíba em Pindamonhangaba da Agência Paulista de Tecnologia dos Agronegócios (APTA) e a Divisão de Agrotecnologia do Centro Pluridisciplinar de Pesquisas Químicas Biológicas e Agrícolas (CPQBA) da UNICAMP. Foi instalado um ensaio experimental a campo em blocos casualizados com cinco acessos e sete repetições. Foram avaliadas a massa fresca e seca da parte aérea, a massa fresca e seca da raiz, o comprimento da maior haste, a área foliar, o índice de espuma e o teor de ácido fáfico das amostras. O acesso I1800 apresentou uma massa seca de raiz, massa seca da parte aérea e área foliar superior aos demais acessos (176,16 g/planta, 7,301 kg/planta, 155,04 cm²). O teor de ácido fáfico não diferiu entre os acessos (0,640-0,366 %/mm) Existe uma correlação positiva entre área foliar e massa seca da parte aérea e entre área foliar e massa seca de raízes entre os cinco acessos.

Palavras-chave: *Hebanthe eriantha*, plantas medicinais, cultivo, ácido fáfico, Amaranthaceae.

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The fáfia (*Hebanthe eriantha*) has been described as the most important product of extractivism in the region of the Paraíba Valley, São Paulo state, Brazil. Silva *et al.* (2007) also verified that extractivism has been causing the species decrease and endangerment in the region.

Hebanthe eriantha, Amaranthaceae family, is a medicinal plant from the Brazilian Atlantic Rain Forest and it is native to all Brazilian regions. Previously named *Pfaffia paniculata*, in Borsch & Pederson (1997) it was renamed to its old botanic name and classified in the genus *Hebanthe*. More recently, with the review of the *Pfaffia* and *Hebanthe* genera, the species was named

Hebanthe eriantha (Marchioretto, 2008). *Hebanthe eriantha* popular name is fáfia, ginseng-brasileiro, picão-de-tropeiro, solidonia, suma, cipó-suma and also corango-açu in the south of Brasil (Smith & Downs, 1972; Marchioretto, 2008). Another species that has similar uses of *Hebanthe eriantha* and also named ginseng-brasileiro and fáfia, the *Pfaffia glomerata*, didn't change its denomination with the genus review.

Traditionally used in indigenous medicine, fáfia is now recognized by its anticancer and stimulating properties. Its roots have triterpenic saponins, and the nortriterpenoid compounds, associated pfaífic acid and pfaífosides, showed inhibiting effect in tumor cell

culture (Nakai *et al.*, 1984). Besides these compounds, the roots also contain alantoin, sitosterol and stigmasterol (Takemoto, 1983; Nishimoto *et al.*, 1984). In traditional communities in the Paraíba Valley, *H. eriantha* is used to control cholesterol (Barros, 2007).

Many studies showed fáfia extracts antitumoral effect of several cell types, but few investigated extracts action mechanism. Another study, also hereafter described, investigated plant roots extract toxicity and its sexual performance stimulate effect.

Alcoholic extract demonstrated analgesic and antiinflammatory effect of rats edema and low acute toxicity (Mazzanti & Braghiroli, 1994). Silva

et al. (2005) studied anticarcinogenic effects of fafia roots extracts and verified its hepatocarcinogenic inhibitor effect in rats and Carneiro *et al.* (2007) demonstrated fafia extract antiangiogenesis effect in rats. Roots extract produced inhibitor effect of Ehrlich tumor and its ascitic form and also had antiinflammatory effect with no toxic effect in rats (Matsuzaki *et al.*, 2003; Matsuzaki *et al.*, 2006).

Pinello *et al.* (2006) investigated the effect cause of Ehrlich tumor by fafia extract and related the increment of macrophages activity being a possible cause of antitumoral action. The antitumoral mechanism action was investigated by Ballas (2000) that found the hidration quality of sick cells by fafia roots extract that could be a possible cause.

Arletti *et al.* (1999) demonstrated stimulated sexual qualities of fafia extract in rats. The extract also increased sexual hormones level (estradiol, progesterone and testosterone) in rats without adverse effects (Oshima & Gu, 2003).

Patent was granted for fafia extract use for falciform anemy treatment and also for aphrodisiac action, and plant extract was the component of a proprietary compound (Corrêa Junior, 2003).

The present study aimed to study fafia evaluating the vegetal characteristics, root production, and saponin and pfaffic acid quantification of different species populations intended to establish the bases for the cultivation of the species and cooperating with the regional production development of the species.

MATERIAL AND METHODS

Initially, a collection of thirty nine accessions collected from Paraiba Valley region, São Paulo state, Brazil was installed and added in Chemical, Biological and Agricultural Pluridisciplinary Research Center of the State University of Campinas (CPQBA) of Unicamp species germplasm collection. For the present study, five accessions from that collection were used. These five accessions were

selected for this study because they showed the best results of seedling. Seed reproduction of *Hebanthe eriantha* is impracticable and rizome reproduction didn't offer all needed seedlings, therefore, cutting reproduction was the better option. Two accessions were collected from Paraiba Valley (G23 and G42) and another three accessions (I1800, I565 and I1801) were collected by the researcher Ílio Montanari Jr. in Nazaré Paulista (SP), Monte Verde (SP) and Rio de Janeiro (RJ), respectively.

Propagation of cuttings was carried out in a greenhouse with a 50% shading screen and microaspiration irrigation. Cuttings with three until five shoots and 14 to 18 cm of length were cut of plant stems conform shrub cuttings production recommended by Hartmann & Kester (1990). Stem cuttings were applied in powder 20% ANA (naphthalene acetic acid/Nafusako 20). Soon afterwards took place the planting in plastic tubets (13,5 x 3,5 cm) containing Gioplant® substratum. Seedlings were planted in the soil after three months.

The experiment was a combination of five accessions and seven replications in a completely random block design, and it was carried out at the experimental area of CPQBA. There were six plants per plots, with four central useful plants. The plant spacing was 2,0 m and each plant used area was 9,45 m². Blocks A, B, C, D and E were installed on December 11th, 2007, and blocks F and G were installed on January 15th, 2008. At planting time, organic fertilization using 250 g of Provaso® compost per plant was done to improve soil physical structure, while its chemical analyses showed adequate essential nutrients levels. Provaso® compost had 15% of organic carbon, C/N relation of 18/1, 1% of nitrogen, pH of 6, CTC of 300, CTC/C relation of 20 and 45% of humidity.

The harvest of six blocks began when plants were 16 months old, and the another block was harvested at 17 months.

The length of the longest stem of the plants measurement was taken from the plant base until the last bud of the longest stem.

The foliar area was taken from five

leaves from the plant base following Araújo (2007). This measurement was taken from one plant per plot. The foliar area was measured using a LI-COR 3100 digital equipment with direct reading in cm².

To obtain aerial part weighing, fresh harvested aerial part was put in nylon screened bags, weighed and after placed in a drying cabinet for the drying. The drying cabinet was set under constant temperature at 45°C using a forced ventilation system (Falkenberg *et al.*, 2003). After the drying, the material with 6 to 8% of humidity was weighed again.

The collected roots were washed under a water jet to clean the soil on them. After the washing, the roots were weighed and ground in a food processor. Then, the roots were put in a drying cabinet for 24 hours to dry, after they were weighed again (Sonaglio *et al.*, 2003; Falkenberg *et al.*, 2003).

The saponins were determined by the Foam Index described by Costa (1982). 0,5 g of ground root was weighed, 100 mL of distilled water was added and then it was boiled for five minutes. After cooling, the extract was filtered through cotton and the volume completed until 100 mL. A series of ten dilutions was prepared in assay tubes, using 1 to 10 mL of extract per tube, and the volume was completed with distilled water until reaching 10 mL. The tubes were shaken vertically for fifteen seconds and the samples were kept at rest for fifteen minutes. The foam index was determined based on the tube dilution where there was a 1-cm persistent foam ring. The final calculation was made according to WHO (1992).

For the quantification of the total pfaffic acid present in fafia roots, a high-efficiency liquid chromatography technique combined with diode array detection and selective mass detection (LC-DAD-MS) was used, according to Souza (2011) methodology described.

Due to the commercial unavailability, the analytical standard of pfaffic acid was obtained in laboratory by a fractional and purification process of fafia root extracts, previously submitted to acid hydrolysis. The identification of the isolated and purified compound

was confirmed by RMN and mass spectrometry and the chromatographical purity was considered (Souza, 2011).

The samples were processed in a grinder and dried until they became a homogeneous powder; afterwards, the powder was submitted to extraction in hydroalcoholic means and to acid hydrolysis.

The hydrolyzed extract was submitted to an ethyl acetate reaction, then it was concentrated in a rota-evaporator and injected in the chromatography system. The chromatography analyses were done using an Alliance LC-DAD-MS Waters chromatography system equipped with a model 2996 photodiode array detector, a column warming oven, a selective mass detector model 3100, and an Empower software. The separation of the sample constituents was performed in a C-18 100 x 2,1 mm analytical column with 3- μ m particles, using methanol and water as eluent. The quantification was done at 215 nm, confirming the selectivity of the mass detector method.

In the statistical analysis, average comparisons were analysed by Tukey's test at the 5% probability level, using SAS program (1997).

RESULTS AND DISCUSSION

The accessions of this germplasm collection range from 0.40 to 1.0 meters of height, and they do not reach the height of 1.80 m related by Marchioretto (2008). Fafia is a climbing plant, so, the length of the longest stem of the plant doesn't mean its height, but its lateral expansion capacity. The harvested roots

had a maximum diameter of 3 cm and they were very thin. Accession I1800 had the thickest roots.

According to the information given by one of the collectors (Mr. Jose Donizete da Silva) that helped collect the material and that has worked with the species for twenty-five years, fafia shows a physiognomic type: it has big large oval leaves and thick yellowish roots that grow parallel to the soil. This is the most common type in the region. Accessions G23, G42 and I565 present this physiognomic type. Accession I1800 is robust, has light green oval leaves and white roots, and I1801 is a small semi-erect type, little vigorous with reddish leaves and white roots.

Accession I1800 had a different aspect for its roots that differed from the other accessions. After harvested and cleaned, the roots became darker with little dark spots on them, and they turned grey after processed, differing from others found at commercial ones that is light yellow. The other accessions present typical species root coloring. The roots had sweet smell after processed and the changing color indicated an oxidation process. The roots were very fibrous.

About aerial part dry weight, accession I1800 showed the greatest aerial part dry weight of all accessions (Table 1 and Figure 1A). Accessions G42, I565, G23 and I1801 showed a similar aerial part dry weight. Accession 1800 aerial part dry weight average was four times greater than the average of the other accessions.

About root dry weight, accession I1800 showed the greatest root weight

of all accessions (Table 1 and Figure 1B). Its average production by plant, of 176.16 g plant⁻¹ was 89.2% higher than the second one, accession G42 (93.09 g plant⁻¹). Accessions G42, I565, G23 and I1801 showed similar root production, with production average of 93.09; 78.7; 66.81 and 47.81g/plant, respectively. The accessions were harvested at sixteen and seventeen months. The dried root production average of accession 1800, considering the 2x4-m spacing used in the experiment, is equivalent to a production of 220.2 kg of dried root ha⁻¹. The study by Montanari Junior (1999) determined the productivity of three clones of *Pfaffia paniculata* and verified that the clones Rio de Janeiro, Mogi das Cruzes and Nazaré Paulista produced 43.8; 52.4 and 135.5 g of dried rootplant⁻¹. A 1x1-m spacing was used and the harvest was done when the plants were fourteen months old. The results of Rio de Janeiro and Moji das Cruzes clones did not differ among themselves, but differed from Nazaré Paulista clone results. The Rio de Janeiro clone is accession I1801, and the clone Nazaré Paulista is accession I1800. In the present study, Nazaré Paulista clone showed greater averages, but it was lower than one related by Montanari Junior (1999).

For aerial part of biomass, accession I1800 also showed the greatest weight of all accessions (Table 1 and Figure 1A). Accessions G42, I565, G23 and I1801 showed a similar aerial part dry weight. The roots of accession I1800 have a different aspect from those ones that are commercialized by the collectors from the Paraíba Valley region. The commercialized roots have

Table 1. Average of agronomical and chemical data of five accessions of fafia (*Hebanthe eriantha*) [valores médios de resultados agronomicos e quimicos de fafia (*Hebanthe eriantha*)]. Botucatu, UNESP, 2009.

Accessions	Dry weight of aerial part (kg/plant)	Root dry weight (g/plant)	Length of the longest stem (cm)	Foliar área (cm ²)	Saponin level (foam index)	Total pfaffic acid quantity (%/mm)
I1800	7.301 a (±3.97)	176.16 a (±78.85)	279.5 a (±45.46)	155.040 a	181.31 ab	0.632 a (±0.12)
G42	1.666 b (±0.82)	93.09 b (±49.25)	172.5 c (±45.29)	67.525 c	211.12 ab	0.366 a (±0.07)
I565	1.466 b (±0.35)	78.70 b (±41.49)	233.6 ab (±31.89)	45.455 c	208.35 ab	0.520 a
G23	1.102 b (±0.34)	66.81 b (±32.09)	203.9 bc (±54.54)	57.620 c	256.67 a	0.570 a (±0.07)
I1801	0.870 b (±0.48)	47.81 b (±23.99)	110.7 d (±24.04)	105.104 b	168.06 b	0.640 a (±0.10)

Averages followed by the same letter in the column do not significantly differ in Tukey's test at 5% of probability (valores médios seguidos de mesma letra na coluna não diferem entre si, teste Tukey, 5%).

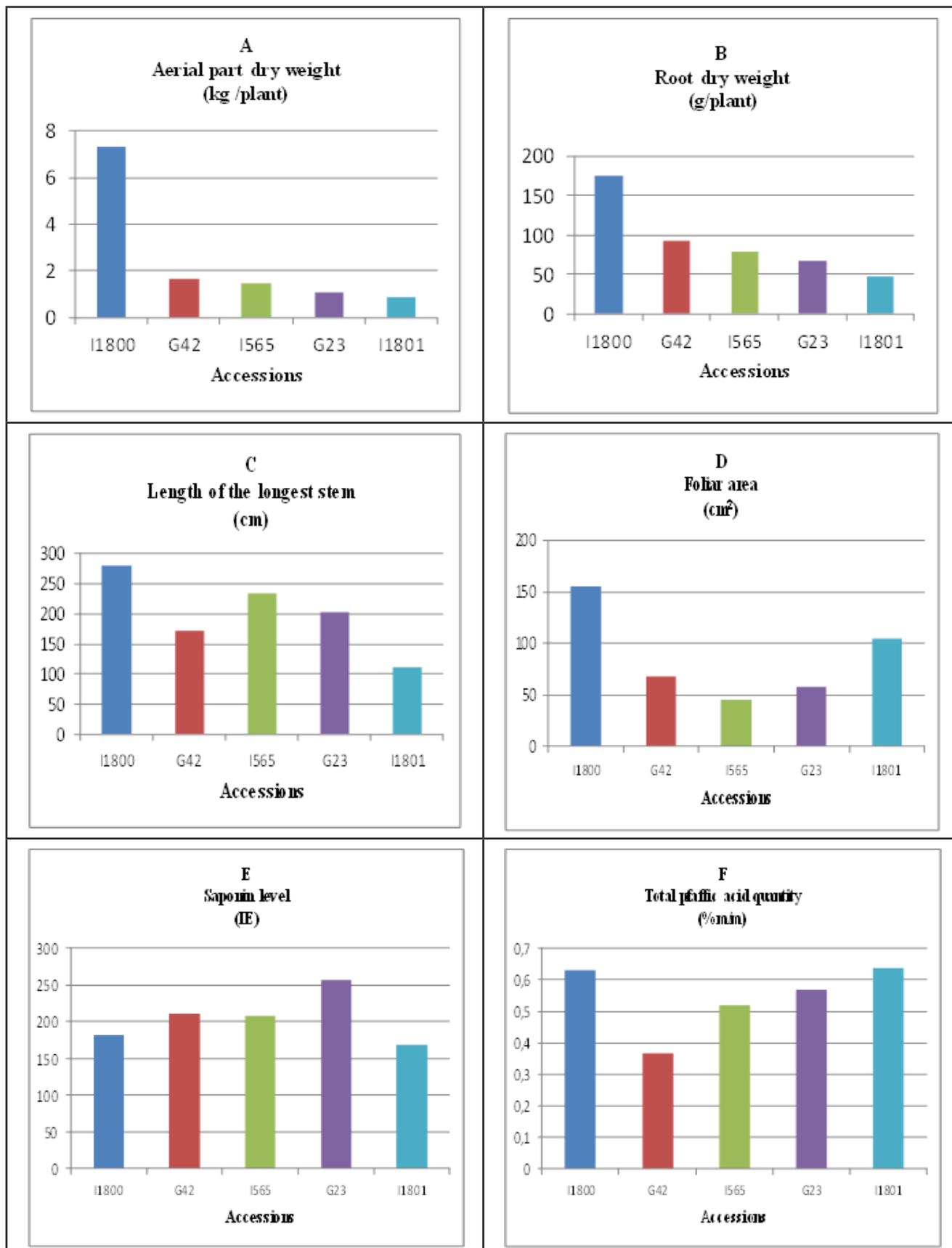


Figure 1. Agronomical and chemical data graphics of five accessions of fãfia (*Hebanthe eriantha*) [(gráficos com dados agronomicos e quimicos de cinco acessos de fãfia (*Hebanthe eriantha*)]. Botucatu, UNESP, 2009.

a yellowish color when turned into a powder. Accession I1800 plants have white roots that become grey when freshly ground. The powder also has a grayish color. The roots and the aerial part of accession I1800 are similar to another *Hebanthe* species, the *Hebanthe pulverulenta* whose roots become dark when processed and present vigorous aerial part, too. The difference of the two species is that *H. pulverulenta* has pulverulent trichomes and ramoso-verticilado on branches, petiole and adaxial-abaxial leaves face, and for *H. eriantha*, abaxial face varies from sub-hairy to slightly hairy and trichomes are estrigosos, articulated (Marchioretto, 2008).

Accession I1800 showed the greatest average for stem length, 279.52cm (Table 1 and Figure 1C), but did not differ from accession I565. This length of the longest stem can help to calculate the spacing and project the horizontal support, but there is no relation with biomass production of the aerial part, because accession I1800 produced five times more the dry weight of aerial part than accession I565. Accession I1801, besides having the shortest stem length, is a semi-climbing plant, like a small shrub when it is in the initial development.

For foliar area, accession I1800 showed the greatest foliar area of all accessions. Accession I1801 showed the second greatest foliar area and accessions G23, G42 and I565 did not differ (Table 1; Figure 1D). This occurred because accession I1800 and I1801 have oval to elliptical leaves, while the other accessions have elliptical to lanceolate leaves.

Regarding the vegetal characteristics, accession I1800 stands out because it shows the greatest aerial biomass production, the biggest stem length and foliar area when compared to the other accessions. Accessions G23, G42, I565 and I1801 have similar vegetal characteristics.

The results of the foam index indicated that accession G23 showed the greatest average (IE=256.67), differing statistically from accession I1801 that showed the smaller average (168.06). The other accessions didn't differ from

accessions G23 and I1801 (Table 1; Figure 1E). The results differed from the ones obtained by Vigo *et al.* (2004) that used the same foam index method and obtained the index 500 ± 0 for fafia.

Regarding the total pfaffic acid quantities, (Table 1 and Figure 1F), the accessions did not show differences among themselves. The study by Nakai *et al.* (1984) in relation to the extraction and determination of compounds of *Pfaffia paniculata* showed 5 g of saponins in 2 kg of dried roots. This is an amount of 0.25% of saponins. However, there are not reference studies of pfaffic acid quantification for *Hebanthe eriantha* yet. As for the other plants that are related to fafia, for example, the *Panax ginseng*, the quantities of triterpenic saponins range from 0.5 to 3% (Schenkel, 2003). For β -ecdisona, present in *Pfaffia glomerata* according to a study by Corrêa Junior (2003), an average quantity of 0.29% was obtained, but other studies reported obtained quantities ranging from 0.64% to 0.76%.

The variables foliar area/root dry weight showed a Pearson's Correlation Coefficient (r) of 0.55 and 0.77 respectively and there was a significant positive correlation to the five accessions of fafia. There was also a significant correlation between aerial part weight/root dry weight, showing a value of $r=0,84$. There was not a significant correlation between the other variables.

Montanari Junior (2005) and Nascimento *et al.* (2007) also found a positive correlation between aerial part characteristics and roots production per plant of *Pfaffia glomerata* accessions. According to Montanari Junior (2005), it means that if roots are the interest, it's possible select good plants that showed good aerial development, and so, it's not necessary a destructive harvest of the plants.

The data from the aerial part dry weight and the length of the longest stem of the accessions indicated the best spacing for the species. A support for this species is important to reasonably exploit the agricultural area, because the stem length average is 279.5 cm in accession I1800, and 233.6 cm in accession I565. A 1.5-m inter-row spacing and 1,0-m spacing between

plants using horizontal support is recommended.

The species presents rusticity and a satisfactory vegetal development with low external inputs. Some plants showed root rotting. Accession I1800 that has a robust vegetal development presented signs of herbivory, but did not decrease production. Herbivory did not happen in other accessions. Although the plant presents rusticity, the species has fragile stems that can break easily during agricultural management.

The most adequate harvest time should be after 16 months which was the one used in this study, because some roots were still thin. Some plants that were harvested at 2 years of age and were not in the experiment, showed much thicker roots.

The root preparation must be made with fresh roots, when they still allow handling. After drying, the manipulation is not possible anymore because the roots have a lot of fibers, making chopping and grinding difficult. For chopping and grinding of a large quantity of material, the use of resistant and robust equipment is recommended.

The present study showed that for future studies of the species, a recommend of news propagation tests is interesting.

The results about dry weight of aerial part and length of the longer branch of the accesses give some indications over the best space for this species. The staking for the species is desirable for a rational use of agricultural area, since the length of the stems can reach on average 279.5 in 1800 and 233.6 in access 565. A spacing of 1.5 meters between rows and 1.0 m between plants with staking may be indicated.

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