## LUMINOSITY LEVELS AND SOIL COMPOSITION INFLUENCE THE GROWTH OF IPECACUANHA

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#### ABSTRACT

*Carapichea ipecacuanha* is a medicinal plant that produces emetine and cephalin. It is on the list of species vulnerable to extinction. The objective was to verify how different substrate compositions and shading levels can interfere in the growth and development of ipecac seedlings. The following substrates were used: black soil (BS), black soil and Bokashi® (BS + B), black soil, sand and Bokashi® (organic fertilizer) (BS + B + S) and black soil and sand (BS + S), and different shading levels (50, 70 and 90 %). The parameters height, number of leaves, diameter and leaf area were analyzed. The shading levels had greater effect in the growth and development when compared to the substrate composition differentiation. Depending on the period, the plants in 50% showed better growth, however, between the months of November to January, these plants showed symptoms of excess of light and temperature.

Keywords: Bokashi®, ipeca, alkaloids, shading, substrate

# NÍVEIS DE LUMINOSIDADE E COMPOSIÇÃO DO SOLO INFLUENCIAM NO CRESCIMENTO DE IPECACUANHA

#### **RESUMO**

*Carapichea ipecacuanha* é uma planta medicinal que produz emetina e cefalina. Está na lista das espécies vulneráveis à extinção. Objetivou-se verificar como diferentes composições de substratos e níveis de sombreamento podem interferir no crescimento e desenvolvimento de mudas

de ipecacuanha. Foram utilizadas as seguintes combinações de susbtratos: Terra preta (TP), terra preta e Bokashi® (adubo orgânico) (TP+B), terra preta, areia e Bokashi® (TP + B + A) e terra preta e areia (TP + A), e diferentes níveis de sombreamento (50, 70 e 90 %). Foram analisados os parâmetros altura, número de folhas, diâmetro, área foliar. O nível de sombreamento afetou mais o crescimento e desenvolvimento quando comparado à diferenciação da composição dos substratos. Dependendo do período, as plantas em 50% apresentaram melhor crescimento, porém, entre os meses de novembro e janeiro, essas plantas apresentaram sintomas do excesso de luminosidade e de temperatura.

Palavras-chave: Bokashi®, ipeca, alcaloides, sombreamento, substrato

#### **INTRODUCTION**

The ipecacuanha [*Carapichea ipecacuanha* (Brot.) L. Andersson], an undershrub from the Rubiaceae family, is also commonly known as ipeca or poia. Recognized historically and worldly as a medicinal plant, it is originated from humid and gloomy tropical forests in the South and Central America. In Brazil, it is most abundant in the states located in the Amazon Rainforest region, having a higher presence in Mato Grosso (LAMEIRA, 2002; FERREIRA JÚNIOR et al., 2012).

The pharmacological nature of the ipeca comes from its roots, that are carriers of isoquinoline alkaloids, emetine and cephaline, with emetic potential (LAMEIRA, 2002; HAN et al., 2013; LÓPEZ et al., 2020). A big economic value is attached to the ipecacuanha for having anti-inflammatory properties and for the possibility of being used in the treatment against amoebiasis, respiratory diseases as asthma and bronchitis (LAMEIRA 2002; GARCIA et al., 2005; VIEGAS et al., 2014). Furthermore, the emetine has antiviral properties, being target of studies for the treatment of current sickness as Zika, Ebola and COVID-19 (VALADÃO et al. 2015; YANG et al. 2018; BLEASEL & PETERSON, 2020).

Factors such as intense irregular extractivism in the last two centuries and the opening of agricultural frontiers, contribute for the reduction of its area of natural occurrence, adding to that, the physiological characteristics inherent to the plant, as its slow growth and inefficient sexual reproduction, guided the ipecacuanha to be classified as an endangered species (ZAPPI et al., 2013) and in risk of genetic erosion (LAMEIRA, 2002; TEIXEIRA et al., 2012; SILVA et al., 2019).

Thus, studies that verify how this species grow and develop in artificial environments are important, in the search for maintenance of this species in its natural habitat and consequently in a better recommendation of its cultivation to farmers that commercialize this species, in a way that enables them to produce in commercial scale in environments that come close to their habitat, but artificial, in greenhouses with shading or different sources of substrate, enabling to add knowledge about this species, seeking the sustainability of the production chain.

Therefore, the present study aimed to verify how different substrate compositions and levels of shading can interfere in the growth and development of ipecacuanha seedlings, for a determined period, in the climatic conditions of Niterói, Rio de Janeiro State, Brazil.

#### MATERIAL AND METHODS

The plant material used (roots) in the experiment were from Banco Ativo de Germoplasma of ipecacuanha *ex-situ* from Horto de Plantas Medicinais da Embrapa Amazônia Oriental, from the county of Belém, Pará State, with the experiment being held in greenhouses from the project "Banco Ativo de Germoplasma de Ipecacuanha", in the Gragoatá Campus at Universidade Federal Fluminense, where the coordinates are latitude of 22°54'00" S, longitude of 43°08'00" W and altitude of 8 meters. The region has an Aw climate, (Köppen classification), tropical climate with dry winters and rainy summers, with an annual average temperature of 23 °C and average annual precipitation of 1,200 mm and the experimental period was between June of 2014 and January of 2015.

For the beginning of the experiment, it was done a reproduction by stakes (roots) and for the sprouting, the roots were allocated in trays containing sand substrate, as described by Ribeiro et al. (2017). After 120 days of the beginning of sprouting, when the species presented from three to four leaves, they were transferred to polyethylene vases of eight liters, separated in four different treatments according to the types of substrates: black soil (BS); black soil and Bokashi® (BS + B) with 7.5 g/m<sup>2</sup> of Bokashi® per vase; black soil, sand and Bokashi® (BS + B + S), in the proportion of 2:1; and placed under shades with three levels of shading (50, 70 and 90%). Bokashi® was a moistened and fermented mixture of soil, rice husks, vegetable husks and sugar cane molasses.

The plants were irrigated daily, with the use of sprinklers, aiming to maintain the humidity levels necessary for their growth. Three temperature measurements were made, in three different times of day, at 6, 12 and 18 hours and the temperatures were measured with a soil thermometer.

To increase the aeration, every day, between 16h and 9h in the morning, the sides of the shades were raised. In the period of 9h to 16h, in addition of the protection of the shades, a sheet of "Aluminum Duralfoil Thermal Insulator" (ADTI) was also inserted as protection to prevent the incidence of direct solar radiation.

The analysis of height, number of leaves, stem diameter and foliar area were done weekly. For the foliar analysis, obtained through length *versus* width, it was used 10 leaves in each treatment according to Flumignan (2008). Regarding the determination of height and diameter, the transplant depth was standardized, having as criteria the covering of stakes up to the sprout's base, being measured with caliper and ruler graduated in centimeters. In the evaluation of variable diameter, the caliper was positioned in the base of the sprout emission and the values were recorded in millimeters. For the variable height, the ruler was positioned in the base of the sprout emission and measured until the beginning of leaf insertion (TEIXEIRA, 2008).

A chemical analysis was also done for the different substrates used, done by Embrapa Solos, Rio de Janeiro. According to the analysis, independent from the type of treatments, the soil was characterized as eutrophic, with aluminum saturation of 0%, salic for BS and BS +B and saline for BS + B + S and BS + S. The values of pH found were slightly acidic, close to 5,5. After the sprouting and development of the species, a sample was deposited in the herbarium in the Jardim Botânico from Rio de Janeiro (RB 790604).

#### **RESULTS AND DISCUSSION**

When analyzed the interactions between black soil (BS) with the different levels of shading (Fig. 1) it was observed that between the months of May and October, the plants in shading of 50% presented a more positive responses in relation to the shadings of 70 and 90%, when analyzed the height and diameter (Fig. 1A and 1B), besides the data in number of leaves and foliar area (Fig. 1C e 1D). However, between the months of November of 2014 and January of 2015, the plants under 50% of shading presented symptoms of excess of light and temperature, with yellowish leaves. In the other shadings there was a low growth in the months that preceded the transplanting, showing regrowth starting in the month of November.

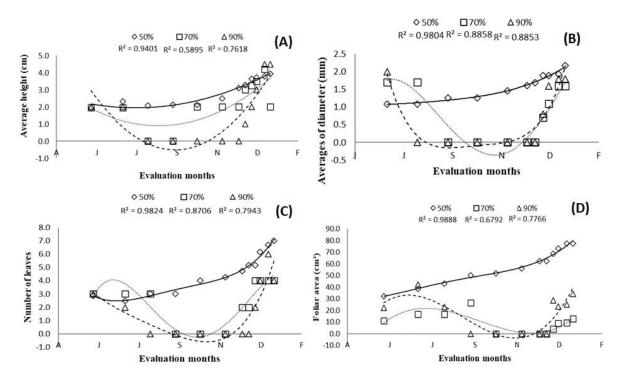


Figure 1. Parameters of growth of *Carapichea ipecacuanha* when cultivated in the black soil (BS) substrate and shadings of 50, 70 and 90%, in the years of 2014/15. A. Average height;
B. Average of diameter; C. Number of leaves; D. Foliar area.

In the cultivation with controlled shading, where the incidence of direct radiation was avoided between 9 and 16 hours, in the months of November of 2014 and January of 2015, the plants cultivated in the three shadings showed more blooming in relation to the same treatments without this control. Comparing the ambiance temperatures where the incidence of direct radiation was controlled and the other greenhouses, there was a difference in temperature around  $4^{\circ}$ C, which might have contributed to a better development in the shading of 50%.

In the work done by Coelho (2013), the plants of ipecacuanha also went by a process of yellowing, break and leaf blight, fact that occurred due to stakes damage. This evidence comes to confirm the informations from Ocampos (2006), which affirms that the ipecacuanha is a species of high temperatures and, therefore, can be planted in a shaded location for a long period, as long as it is maintained in an average temperature, higher than 25 and lower than 28°C. In accordance with the data observed, the plants in the shading of 70 and 90% felt this temperature variation more than those in the 50% shading.

The values of the average temperatures of all substrates for the three shadings in the three main hours of the day (6h, 12h and 18h) were higher in the shading of 90%, being 22.36, 28.75 and

27.82°C, respectively (data not shown). This is due to information already mentioned by Mattos (2001), and Andrade et al. (2011) in which the air temperature in cultivation systems protected by plastic cover tend to be more elevated in the daytime and equal or lower in the nighttime.

Different degrees of luminosity cause, in general, morphological, and physiological changes in the plant, in which the degree of adaptation is dictated by particular characteristics in each species in interaction with its environment (SCALON et al., 2003; KLEINWÄCHTER & SELMAR, 2015). The ipecacuanha morphological response, and the growth were more efficient when submitted to the shading of 50%, considering the experimental region, in the Rio de Janeiro State, between the periods of May and November.

The parameters of growth of plants of ipecacuanha when cultivated in the substrate of black soil plus Bokashi® (BS + B) depending on the shading of 50, 70 and 90%, showed that the level of 50% shading had a gradual growth in relation to the shading levels of 90 and 70%, getting to the end of evaluation with an average height of 4.21 cm. The shading of 70% resulted in a plant height of 2.00 cm, as the representatives of this treatment dried up and re-sprouted during the evaluation period. In the shading of 90% the areal part of the plant went into senescence, remaining only the underground part (Fig. 2).

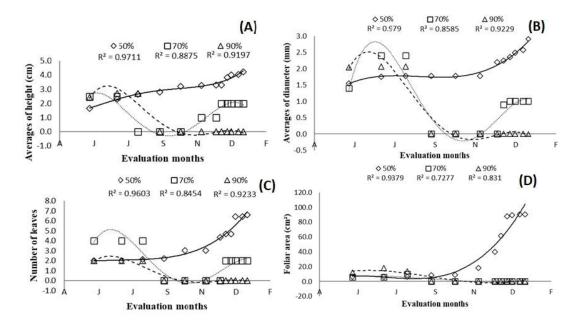


Figure 2. Growth parameters of *Carapichea ipecacuanha* when cultivated with the substrate black soil plus Bokashi® (BS + B) and shadings of 50, 70 and 90%, in the years of 2014/15.
A. Averages of height; B. Averages of diameter; C. Number of leaves; D. Foliar area.

This same response of the ipecacuanha was also verified by Garcia et al. (2005), who observed, that in the period of mild temperature, plants of ipecacuanha devoid of leaves (young or mature), presented only aerial branches that, with the passage of time, became dry. In some times of the analyzed period, in this work, it was observed an emission of new buddings from underground branches, however the growth of these buddings was soon interrupted, going into senescence in the sequence. The same was observed in the shading of 90%, which was similar to what Garcia et al. (2005) observed, when in condition of undergrowth, a percentage of only 3.5% of solar radiation got to the plants, showing little luminosity available.

In relation to the diameter, the applied condition was the same as the height parameter, getting to the end of the evaluation with values of 2.91 mm for the shading of 50 % (Fig. 2A e 2B). As for the shading of 70%, at the end of the experiment the plants presented averages around 1 mm diameter. The relative height mentioned refers to the cycle of eight months of growth, since the species stabilizes in the period of two years, reaching an average height of 30 cm (LAMEIRA, 2002).

The species when compared in substrate black soil plus Bokashi® (BS + B) presented an average of seven leaves for the shading of 50% and two leaves for the shading of 70% (Fig. 2C). The same values were observed in the treatment with the substrate black soil (Fig. 1C), indicating a higher influence from the shading than the substrate. In relation to the foliar area (Fig. 2D), the lower values were observed for the shading of 90%, which lost all its experimental portion, and for the shading of 70%, that obtained a 7.56 cm<sup>2</sup> of foliar area. The higher values were observed in the shading of 50%, which reached the value of 135.50 cm<sup>2</sup> of foliar area.

Through the calculus of difference in foliar area on the experiments, it was observed that the species responded in an uneven way when submitted to different levels of radiation. For the environmental conditions of the region where the experiment was done, it was observed a modification in leaves in relation to the dimension when exposed to the shading of 50%, besides the alteration in its coloration, tending to a more yellowish color. As for the plants that were in the shading of 70%, presented a dark green coloration. According to Dickson (2000), the leaf is the organ that suffers more anatomical modifications in relation to the luminosity variations of a determined environment. Castro et al. (2009) also highlight that neighboring taxonomic groups that grow in different environmental conditions can present differences, especially in relation to foliar anatomy.

For the substrate black soil plus Bokashi<sup>®</sup> and sand (BS + B + S), in relation to the height and diameter, there was a distinction between the values found in different levels in shading at the end of the evaluation (Fig. 3A and 3B). The average of the values obtained in the shadings of 90, 70 and 50%, respectively, was of 0, 3.48 and 4.75 cm for the height and of 0, 2.1 and 2.7 mm for the diameter.

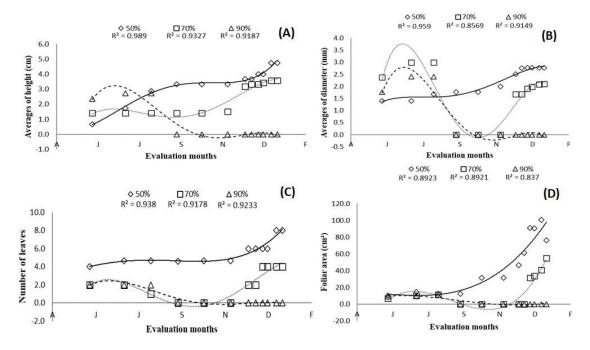


Figure 3. Growth Parameters for *Carapichea ipecacuanha* when cultivated with substrate of black soil plus Bokashi® plus sand (BS + B + S) and shadings of 50, 70 and 90%, in the years of 2014/15. A. Averages of height; B. Averages of diameter; C. Number of leaves; D. Foliar area.

As for the values in number of leaves and foliar area (cm<sup>2</sup>), (Fig. 3 C and 3 D), for the cultivation in the substrate black soil plus Bokashi® and sand (BS + B + S), it was verified an average number of eight leaves for the shading of 50% and of four leaves for the shading of 70%. The same values were observed in the treatment with the substrate black soil (Fig 1C), it was observed again a higher influence of the shading than the substrate. As for the foliar area, the lower values were observed for the shading of 90%, that lost all its experimental portion, and the shading of 70%, that obtained a 55.11 cm<sup>2</sup> of foliar area. The higher values were observed in the shading of 50%, with a foliar area of 100.73 cm<sup>2</sup>.

Ribeiro et al. (2019a), studying this species, also verified that the plants in the shading of 50% presented a rapid growth, however, in the shading of 90% there was an increase in values for being a species of shaded environment, being dependent of the period of the year (season) in which they were analyzed. Nogueira et al. (2021) also verified that for this species, the shading of 90% for all the seasons of the year, was the most adequate for the photochemical activity of photosynthesis, however the shading of 50% presented a lower performance index in all the seasons, when compared to the control, when seeking an indication of artificial shading for this species, based on results of the photochemical activity of photosynthesis.

For the cultivation in the substrate black soil plus sand (BS + S), in relation to the height and the diameter (Fig. 4A and 4B), it was verified a distinction between the values of the different levels of shading at the end of the evaluation. The averages obtained, in the shadings of 90, 70 and 50%, respectively was of 4.66, 0.00 and 6.12 cm for the height and 2.2, 0.00 and 2.67 mm for the diameter. As for the results of number of leaves and foliar area (cm<sup>2</sup>) (Fig. 4C and 4D) the values demonstrated that the shading of 50% stood out in relation to the other treatments, presenting in the final evaluation, in average, six leaves and a foliar area of 1178.52 cm<sup>2</sup>. In the shading of 90%, the values were, in average, of four leaves and 94.99 cm<sup>2</sup> of foliar area and in the shading of 70% it plants with leaves were not observed.

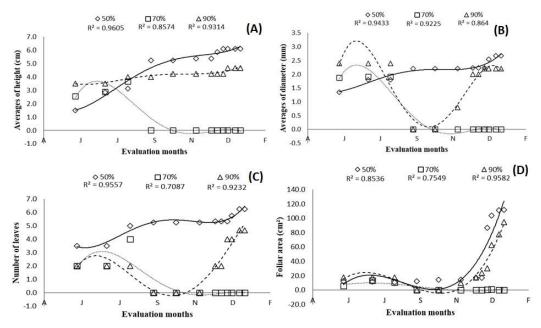


Figure 4. Growth parameters of *Carapichea ipecacuanha* when cultivated with substrate black soil plus sand (BS + S) and shadings of 50, 70 and 90%, in the years of 2014/15. A. Averages of height; B. Averages if diameter; C. Number of leaves; D. Foliar area.

In relation to the minimum and maximum values of soil temperature observed in the shadings of 90, 70 and 50% were, in average, of 19°C for all shading levels and 33, 31 and 31°C, respectively for each one. The lower temperature levels were observed in the months of June and July and the higher ones in the months of December and January, coinciding with the seasons of winter and summer for the region.

Based on the data above, it is observed how each vital process, namely, germination and vegetative growth, were adapted to a temperature range, where greater growth was only achieved when the various environmental factors that influence the plant's metabolism were optimized. Hence, it is possible to notice that the temperature had a direct influence in the growth and development of this species, as the temperature in the region declines, the interfere in several biological processes influence the growth and the development of this species.

The more adequate growth, by cellular division requires a quantity of considerable heat, where the optimal temperature for cellular division is approximately 30°C for most herbaceous plants, on the other hand, the cellular differentiation can be done in low temperatures, even if slowly (COSTA et al., 2016). The ipecacuanha, as an herbaceous species, was impaired when exposed to low temperatures in the month of July and September. On the other hand, when exposed to higher temperatures associated with an adequate shading, presented a positive growth.

The ipecacuanha is a species of shaded environment and when submitted to lower radiation, as done in the experiment (90%), it was obtained a foliar area higher than the one obtained in other shadings (50 and 70%), as foreseen, because when species are cultivated in environments with more shading that the ideal level (70%), which is considered control (Lameira, 2002), there is an increase in the foliar area, providing a higher surface for photon capture (TAIZ et al., 2017). This is important, because generally the increase in foliar area and increase in number of leaves are factors that directly influence the productivity, because it determines the interception of light, however, it is also important to verify that this or other factors also influence the production of isoquinoline alkaloids. This was also verified when this species was cultivated in different levels of shading an in relation to the seasons of the year, where Santos et al. (2020) and collaborators, found a higher content of cephaline in the winter under 70% shading, while for the emetine was registered in the spring, in 90% shading. The autumn (shading of 70%) was the only season in which the quantity of emetine and cephaline were similar.

After eight months from the beginning of the experiment, by removing the roots of ipecacuanha as specimens in each substrate, it was found that the roots of plants in shadings of 50% outperformed the other levels of shading, when compared the root growth process. The roots under the shading of 50%, independent of the substrate, obtained an average of 7.5 cm of length. As for the levels of shading of 70 and 90%, the average values were of 3 cm of length (Fig. 5), which is reported in literature, roots with greater average diameter, greater mass and root volume that were collected in tumbleweed, which were exposed to a higher level of luminosity (GARCIA et al., 2005).

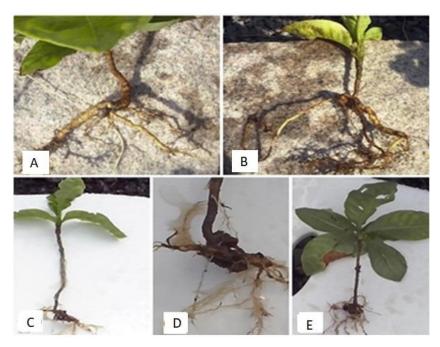


Figure 5. Roots of *Carapichea ipecacuanha* when cultivated under different shading levels. A and B. Shading of 50%; C and D. Shading of 70%; E. Shading of 90%.

Another factor observed in relation to the growth habits is that when this species reaches the height of 30 cm, the stem bends towards the soil, becoming most times a replanting and forming false rhizomes that emit nots and later lateral roots that transform into tuberous roots rich in reserved substances, as also reported by Pinto (1972). After this process, it was verified that a sprouting occurred on this stem, in the apical parts, median and basal and in the roots formed (Fig. 6).



Figure 6. Sprouting surging in various parts of the plant *Carapichea ipecacuanha* body. A. Middle part of the stem; B. Basal part of the stem; C. Formed roots; D. Basal part of the stem.

Thus, in a general manner, for the adequate growth and development of the seedlings of ipecacuanha, the shading influenced more than the substrate, the same was verified in works with ipecacuanha, when only analyzed the growth and development of seedlings in different shadings, it was also verified better results in the shading of 50%, preferably with thermal isolation, for the lowering of temperature variation, having in mind its natural habitat is one of undergrowth (RIBEIRO et al. 2019b), but this depends on the season of the year.

## CONCLUSION

The level of artificial shading affects growth and development the most in the seedlings of *Carapichea ipecacuanha* than the substrate composition used in this experimental condition. Thus, between the months of May and October, the plants cultivated in the shading of 50% presented a more positive response in relation to growth when compared to the shadings of 70 and 90%. However, between the months of November and January, the plants under 50% of shading presented symptoms of excess luminosity and temperature, presenting morphological changes in the leaf blade, also with a more yellowish coloration than the other levels of shading.

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