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Domestication Test of *Halopegia azurea* (Karl Moritz Schumann) (*Marantaceae*): Preliminary Study on the Rhizome Cuttings

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Abstract: In order to domesticate *H. azurea* (*Halopegia azurea*), acclimatization of rhizome's cuttings and young shoots in farms was tested at Nkol Evodo village from February 2012 to June 2012. The best substrate for propagation was assessed: (0) wetland; (1) sand/wetland; (2) sawdust/wetland; (3) sand/sawdust/wetland. Young shoots' growth was observed in situ for 8 weeks. A significant difference ($P < 0.05$) was observed in the appearance of young shoots and leaves in nursery. The highest average number of young shoots and leaves (0.62 ± 0.09) was found in substrates 0 and 1, the lowest (0.31 ± 0.08) in substrate 3. Substrate 3 showed the lowest number of young leaves (0.41 ± 0.16) and substrate 2 the highest (0.97 ± 0.24). A significant difference in the mortality rate of cuttings ($P < 0.05$) was observed: 68.75% and 53.12% in substrates 2 and 3, respectively. On farm, new leaves and young shoots' appearance showed a highly significant difference. Plants growing in substrates 2 and 3 showed the highest number of leaves. New shoots developed only in plants growing in substrates 0 and 1. These results suggest that it is possible to domesticate *H. azurea* for leaves' production in quantity and quality, and open up better opportunities for its cultivation.

Key words: *H. azurea*, domestication, cuttings, substrate, farm.

1. Introduction

NTFPs (non-timber forest products) serve to enhance the role of trees and forests in improving human well-being because they represent an alternative source of employment and generate significant revenue.

The contribution of NTFPs to household economies and food security is increasingly recognized [1]. However, commercialization of NTFPs is attracting more and more interest and this often leads to over-exploitation [2]. It becomes necessary to identify the conditions for an ecologically sustainable and economically viable operation and to even develop strategies for their conservation and domestication [3]. In central Africa, especially in Cameroon, leaves are harvested for many uses, and harvesting concerns both ligneous and herbaceous plants. *Marantaceae* leaves are among the most widely used leaves of herbaceous plants [4]. They are not edible but play an important role in food preparation, especially the leaves of *H. azurea* (*Halopegia azurea*) [5]. Thanks to their size and lack of active compounds, they are suitable for food packaging [6]. *Marantaceae* are an important family, farmers sell them for the packaging of various products, for the production of wickerwork and for construction of roofs [7]. The CF COPAL (Community Forest of the Cooperative of Lekié's Farmers), site of this study, is home to many natural resources including both timber and NTFPs. The latter can be of animal or vegetable origin and are an important source of livelihood not only for the local people but also for those of urban areas. These products are subject to many threats that can be summarized in one major cause: over-exploitation for

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auto-consumption and/or commercialization. *H. azurea* is under the same pressure in the CF COPAL. The leaves of this species are highly exploited for the packaging of cassava dough leading to the making of a product called cassava baton which is an important source of revenue for the people of this locality [8]. These leaves are also used for the packaging of sun flower seed-pudding and peanut paste. They are also used for the packaging of cassava leaves and fish dishes, as well as many others.

Following the growing demand of the leaves of this species, it is becoming increasingly rare so much so that local communities are forced to go to the most remote areas of the forest to get some and supply in Yaoundé, the capital city located about 60 km from this locality [9]. Other anthropogenic activities such as agriculture, fishing, logging, non-appropriate methods and frequency of harvest couple to climate change also contribute to the scarcity of *Marantaceae*. This study was conducted in CF COPAL in order to find a method of domestication of *H. azurea* which is very popular for its leaves in Cameroon and in the central African sub-region.

2. Materials and Methodology

2.1 Location of the Study Area

The study was carried out in a nursery in Nkol Evodo, one of the neighboring villages of the community forest and head office of the CF COPAL. The area chosen for the experiment has a relatively flat land especially for the nursery, with availability of water at all times (wells), and the area is well secured for the progress of the work. The climate is equatorial with four seasons: a long dry season from mid-December to mid-March, a short rainy season from mid-March to mid-May, a long rainy season from mid-September to mid-November and a short dry season from mid-May to mid-August. The annual average temperature varies between 20 °C and 24 °C. The relative humidity varies between 76% and 84% depending on the season.

2.2 Biological Material

In order to carry out this work, healthy rhizomes of *H. azurea* plants were harvested at Edok assi swampy forest area. The plant material has to be very representative because it contains reserves of nutritive elements essential to the blossoming and the nutrition of young shoots in the early growth stages of the plant. The roots should not have symptoms of nematode attacks (reddish coloration of the woody part of the root or necrotic roots). Rhizomes selected must have multiple nodes.

2.3 Establishment of Experimentation Area

2.3.1 Development of the Nursery

The nursery used consists of a slightly defective acclimatization chassis which was constructed during the project on vegetative propagation, development of nurseries and plantations of *Gnetum africanum* with the support of the CIFOR (Centre for International Forest Research) to the CF COPAL. The chassis was built in 2009.

The operation consisted specifically in the cleaning of the test site and the rebuilding of the chassis in order to be able to maintain a temperature, humidity and moderated light intensity favorable for good development of the cuttings.

2.3.2 Experimental Preparation of Substrates

The various basic materials (wetland or marshy soil, sand, sawdust) are taken separately. Three graduated buckets (10 L) are used to prepare the experimental substrates. For the composition of the substrate of Test 1, 10 L of marshy soil and 10 L of sand are mixed. For Test 2, 10 L of marshy soil and 10 L of sawdust are taken. Finally for the last mixture, the three basic substrates are used in equal portions, that is, 10 L each. The substrate of the control test is not mixed. Once the substrates are obtained in the desired proportions, they are placed in perforated nursery bags to allow the drainage of excess water applied during watering. The bags are then labeled with the dry bark of bamboo and a permanent marker.

2.4 Experimental Setup in the Nursery

The experimental setup in the nursery consists of completely randomized blocks, having as variable the type of substrate, and as experimental unit, bag filled with experimental substrate. Four treatments (substrates) were selected, and each treatment consists of 32 repetitions (bags), making a total of 128 cuttings (bags) for the four substrates.

The four tests carried out are the following (Fig. 1):

- Test 0 (32 bags): control substrate consisting of marshy soils (TM (Terre Marécageuse)) or wetland, soil on which *H. azurea* usually grows;
- Test 1 (32 bags): mixture of fine sand (SA (Sable)) and marshy soils (volume/volume);
- Test 2 (32 bags): mixture of sawdust (SC (Sciure)) and marshy soils (volume/volume);
- Test 3 (32 bags): a mixture of marshy soils, sand and sawdust (volume/volume).

2.5 Disposition in Natural Environment

Young plants used in the natural milieu are coming from cutting tests carried out in the nursery. A total of 24 plants of *H. azurea* were selected and placed in the swamp, that is, eight plants per substrate. The choice of the number of young shoots per culture medium on the field is based on the substrate which has the lowest yield in the nursery (here, eight best plants are selected to harmonize the experiment, the same process is performed for plants of other substrates). The plot is chosen on the basis of the presence of a few tufts of *H. azurea* in this environment which is a swampy area and the availability of the land for experimentation.

2.6 Observations Carried out

Observations and measurements were made weekly for 8 weeks in the nursery as well as on the field. The data collected in the nursery are made according to the substrate: the number of leaves per plant, number of shoots appearing, and the number of dead cuttings. And in the natural milieu, the data collected in the nursery are made according to the number of leaves of each seedling, the number of dead plants, and the number of shoots per plant.

2.7 Data Analysis

The data collected are processed manually. Statistical analyses were performed using the Graphpad Instat Software. Analysis and separation of significant mean were done using Dunnett's multiple comparison tests. And all measured parameters are subjected to analysis of variance at 5% threshold. Microsoft Excel spreadsheet was also used.

3. Results and Discussion

3.1 Nursery Results

Development is defined as the set of qualitative changes in the plant, corresponding to the initiation and the appearance of new organs (roots, stems, leaves, flowers, fruits, and seeds) [10]. Three parameters (leaves, shoots and mortality) related to the development

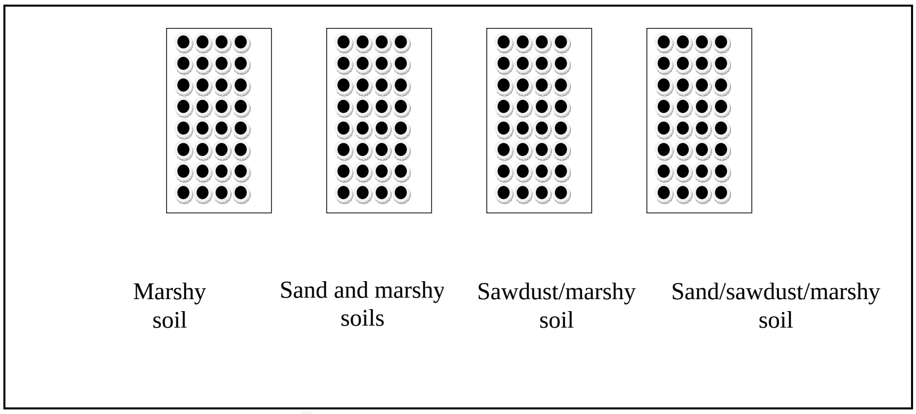

Fig. 1 Diagram of the experimental nursery. ●: experimental unit (plastic bag).

of the plant were used to express the results of the experiment in the nursery.

3.1.1 Influence of the Substrate on the Production of Leaves

Development was followed up by the counting of the newly appearing leaves for each substrate. The ability of the occurrence of new leaves is expressed by the average number of leaves per substrate. In the 6th week, the average number of leaves varies in the four culture media, in the marshy soil (0.76 ± 0.17), the sand/marsh soil mixture (0.65 ± 0.17), the sawdust/marshy soil mixture (0.31 ± 0.12), and finally sand/sawdust/marshy soil mixture (0.56 ± 0.16) (Table 1).

The observed difference is only apparent since the variance analyzes showed no significant difference ($P > 0.05$). However, from the 7th week and 8th week, the sawdust/marshy soil substrate really stands out from the other two recomposed substrates. Statistical analysis of the data shows a significant difference in the production of the number of leaves between the marshy soil substrate and sawdust/marshy soil mixture unlike the two other recomposed substrates.

3.1.2 Influence of the Substrate on the Occurrence of Shoots

The ability of occurrence of shoots is expressed through the average number of shoots per substrate. The high number of shoots is observed in all the different substrates from the first week. From the 1st week to the 6th week, there is a variation in the number of shoots variation between different culture media, but statistical analysis showed no significant difference ($P > 0.05$) between these different culture media at these various dates. In the 7th week, the number of shoots still varies in different culture media, marshy soil (0.62 ± 0.09), sand/marshy soil (0.53 ± 0.09), sand/sawdust/marshy soil (0.47 ± 0.09) (Table 2). It is noted that, after analysis, there is no significant difference between the three culture media. Nevertheless, these three substrates stand out from the sawdust and marshy soil mixture (0.31 ± 0.08). A more detailed analysis of the Dunnett multiple comparisons test showed a significant difference. And this difference is maintained until the 8th week. This explains the effectiveness of marshy soil, sand/wetland substrates, and the sand/sawdust/wetland mixture. As

Table 1 Influence of the substrate on the weekly change in the number of leaves of *H. azurea* in nursery.

Weeks after planting	Average number of leaves					
	3	4	5	6	7	8
TM (control)	0^{a}	0.25 ± 0.07^{a}	0.47 ± 0.13^{a}	0.76 ± 0.17^{a}	0.93 ± 0.23^{a}	1.12 ± 0.28^{a}
TM and SA	0.12 ± 0.06^{a}	0.19 ± 0.13^{a}	0.44 ± 0.13^{a}	0.65 ± 0.17^{a}	0.65 ± 0.12^{a}	0.97 ± 0.24^{a}
TM and SC	0^{a}	0.06 ± 0.04^{a}	0.22 ± 0.09^{a}	0.31 ± 0.12^{a}	$0.31 \pm 0.12^{\mathbf{b}}$	0.41 ± 0.16^{b}
TM-SA and SC	0.09 ± 0.05^{a}	0.19 ± 0.07^{a}	0.41 ± 0.12	0.56 ± 0.16^{a}	0.59 ± 0.16^{a}	0.62 ± 0.17^{a}

TM = marshy soil; SA = sand; SC = sawdust; the averages of each column that are not followed by the same letter in power; ^{a} and ^{b} are statistically identical to the probability level of 5%, no significant differences.

Table 2 Influence of the substrate on the number of emerging shoots of *H. azurea*.

Weeks after planting	Average number of shoots					
	3	4	5	6	7	8
TM (control)	0.75 ± 0.08^{a}	0.78 ± 0.09^{a}	0.66 ± 0.08^{a}	0.62 ± 0.09^{a}	0.62 ± 0.09^{a}	0.62 ± 0.09^{a}
Mixture TM and SA	0.62 ± 0.08^{a}	0.59 ± 0.08^{a}	0.53 ± 0.09^{a}	0.53 ± 0.09^{a}	0.53 ± 0.09^{a}	0.53 ± 0.09^{a}
Mixture TM and SC	0.47 ± 0.08^{a}	0.59 ± 0.09^{a}	0.47 ± 0.09^{a}	0.34 ± 0.08^{a}	$0.31 \pm 0.08^{\mathbf{b}}$	$0.31 \pm 0,08^{b}$
Mixture TM-SA-SC	0.50 ± 0.10^{a}	0.53 ± 0.09^{a}	0.44 ± 0.09^{a}	0.44 ± 0.09^{a}	0.47 ± 0.09^{a}	0.47 ± 0.09^{a}

TM = marshy soil; SA = sand; SC = sawdust; the averages of each column that are not followed by the same letter in power; ^{a} and ^{b} are statistically identical to the probability level of 5%, no significant differences.

for the sawdust/wetland mixture, it appears to be the least performing amongst all culture media.

3.1.3 Influence of the Substrate on the Mortality Rate of Cuttings

Referring on the results obtained 8 weeks after planting, it appears that the mortality rate varies with culture media. It is of 37.5% in the marshy soil substrate, 43.75% in the sand and marshy soil mixture, 53.12% in the sawdust and marshy soil mixture and 68.75% in the sand/sawdust/marshy soil mixture (Fig. 2). These three substrates used clearly stand out from the sawdust and natural soil mixture. Analysis of variance establishes a significant difference ($P < 0.05$). Statistical differences were observed in the 7th and 8th week of the experiment in the nursery.

3.2 Results in the Farm

Plants from different substrates introduced in the natural milieu show differences in behavior based on their substrate of origin in relation with the output (appearance of new leaves and new shoots of *H. azurea)*. All plants survived in the natural milieu.

3.2.1 Influence of the Substrate on Leaves Appearance

From the beginning, right up to the end of the experiment, curves of the weekly evolution of leaves show a fast growth on plants that developed in marshy soils (Fig. 3). The rate of appearance of leaves of plants from the sand/marshy soil mixture is approximately close to those of plants from the marshy soil substrate. However, as from the 3rd week of the planting in the farm, analyses of variance show a significant difference between plants grown on the other two mixtures (sawdust/marshy soil and sand/sawdust/wetland). This difference gradually increases until the end of the experiment. This explains the very low rate of leaf appearance as compared to the plant from the control substrate and the sand/marshy soil mixture.

3.2.2 Influence of the Substrate on the Emergence of New Shoots

The number of air roots is evaluated on the basis of the substrate of origin of *H. azurea* young shoots in the nursery (Fig. 4). The ability of appearance of new

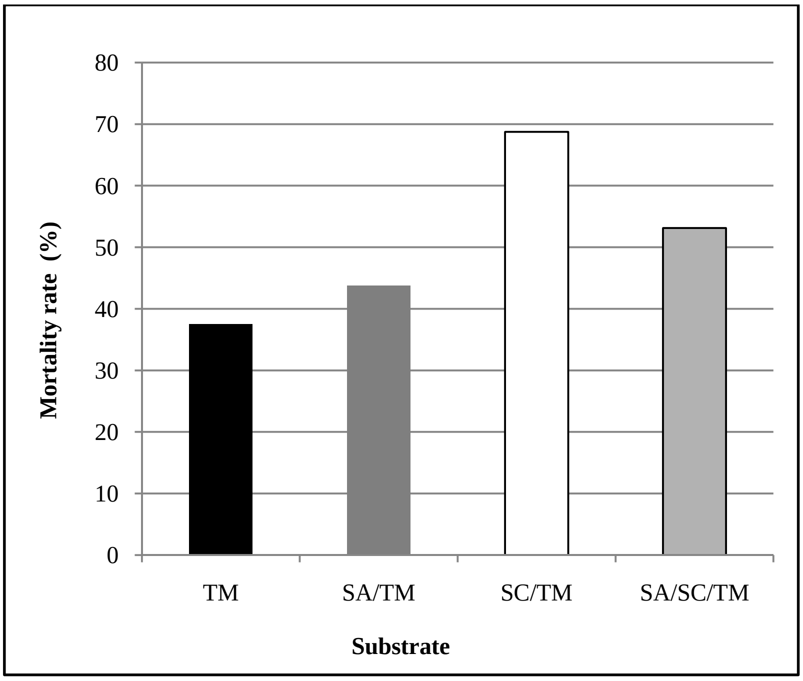

Fig. 2 Influence of the substrate on the mortality rate of cuttings in nursery. TM = marshy soil; SA/TM = sand and marshy soil; SC/TM = sawdust and marshy soil; TM/SA/SC = mixture sand, sawdust and marshy soil.

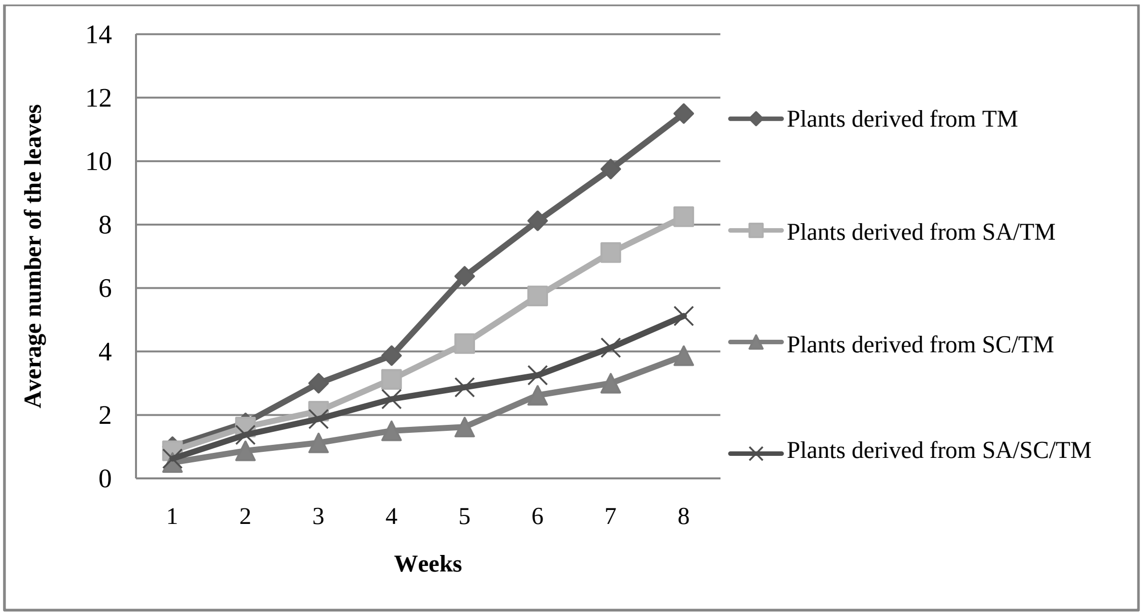

Fig. 3 Influence of the substrate on the appearance of *H. azurea* leaves in the farm. TM = marshy soil; SA/TM = sand and marshy soil; SC/TM = sawdust and marshy soil; TM/SA/SC = mixture sand, sawdust and marshy soil.

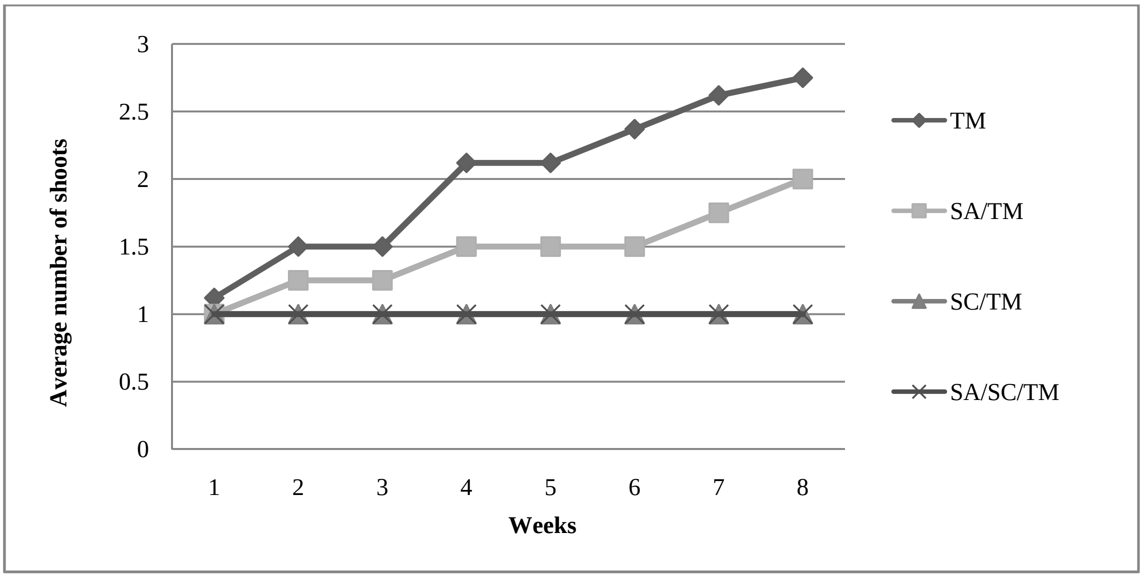

Fig. 4 Influence of the substrate on the appearance of *H. azurea* shoots in the farm. TM = marshy soil; SA/TM = sand and marshy soil; SC/TM = sawdust and marshy soil; TM/SA/SC = mixture sand, sawdust and marshy soil.

shoots is expressed by the average number of shoots per young plant. From the 4th to the 8th week after planting, changes in the average number of shoots of plants from the control substrate show a highly significant difference ($P < 0.001$) between plants from the sawdust/sand and marshy soil/sawdust/marshy soil mixtures. This is explained by a constant number of aerial stems of young plants in the latter two substrates during the experiment in the farm. Concerning plants from Experiment 1, this difference ($P < 0.05$) only appears from the 6th week after planting.

Throughout the period of experimentation in the farm, the number of shoots from young plants of tests 2 and 3 remained constant. However, the rate of appearance of new stems increased for young plants grown in the control and sand/natural soil mixture substrate.

3.3 Discussions

The results at the end of this work show a better performance of the marshy soil substrate on the *H. azurea* cuttings. As for the sand/marshy soil mixture, the results are significantly close to those in the control experiment. This substrate is followed by the sand/sawdust/marshy soil mixture. The lowest performance is obtained from the sawdust/marshy soil mixture, which is different from other culture media.

Data obtained on the influence of the substrate on leaves appearance showed a rapid increase in the number of leaves of plants in the control substrate, approximately similar observations are noticed in the sand/marshy soil mixture. Plants with the lowest number of leave are found in the marshy soil/sawdust substrate. The appearance of buds was more effective in the sand/marshy soil mixture with a significant similarity with the results obtained in the marshy soil substrate unlike the two substrates studied (tests 2 and 3). Substrate quality is an important parameter for the success of the development process of the cuttings [11]. Porous and light substrates have good water retention capacity and at the same time facilitate a good circulation of water and oxygen at the base of the cuttings; this is the case of sawdust [12]. These types of culture medium suggest that the species best suited are those that grow in hydric-saturated conditions like species whose ecological environment of choice is the forest gallery of dry zones [13]. However, in the context of this study, the best performances are obtained in the marshy soil substrate (62.5%) and the sand/marshy soil mixture (56.25%). The latter substrate has low water retention capacity as compared to the sawdust/marshy soil mixture and the sand/sawdust/marshy soil mixtures. Moreover, *H. azurea* is a species that thrives in flooded zones, which can be a sign that it is not suited to these conditions. This confirms the number of shoots and leaves which are very high in the sand/marshy soil mixture in contrast to those in the sawdust/marshy soil mixture (31.25%) at the end of nursery experiment.

The substrate also influences mortality of the cuttings of *H. azurea*. The highest survival rate was recorded in the marshy soil substrate. The results obtained in the sand and marshy soil mixture on the survival of *H. azurea* cuttings are similar to those of the marshy soil substrate (control). This suggests that sand allows for better preservation of cuttings of this species in the nursery. The results corroborate those of Ndam et al. [14] who recommend the sand substrate for the better development of cuttings under glass. And according to Ref. [15], the sand seems to be appropriate for the conservation of fragile cuttings; this is true for a humid environment such as that of the forest. Moreover, the results obtained by Jiofack and Dondjang [16] on the mortality of *Tetracarpidium conophorum* show a relatively high rate of rotten cuttings in sawdust. This is because sawdust unlike the permeability properties of sand retains more water causing the cuttings to get rotten. All these studies performed by these authors confirm the high mortality rate (68.75%) of *H. azurea* in the sawdust/marshy soil mixture.

Ecological conditions necessary for the development of a species are determinant in the natural environment. The plants from the control substrate always show a rapid increase in the number of leaves, and a considerable number of new shoots. Young plants from the sand/marshy soil substrate grow faster than plants from the other two mixtures, and the absence of shoots is noted for the latter two cases. The plants from the sawdust/marshy soil substrate have the least efficient results. The best performance of the marshy soil and sand/marshy soil substrates plants in the nursery is continued in the natural milieu.

The best performance of the sand/marshy soil substrate as compared to the sawdust/marshy soil mixture is contrary to some results obtained in Refs. [11-13], which stress the best performance of sawdust compared to other substrates. This contradiction could be explained by the fact that the used sawdust had not yet reached a state of advanced mineralization, furthermore, the fine sand that was used during experiments was extracted from a swampy area, which may bring the results of this culture medium close to those of marshy soil substrate. Finally, these results may also be due to the origin of the rhizome and the specific characteristics of each species.

4. Conclusions

To make a preliminary test to *H. azurea* culture, tests of domestication from rhizome cuttings were performed, despite many difficulties related to obtaining cuttings and scarcity of the plant cuttings. In light of the information obtained during the experiment, it appears that the domestication of *H. azurea* is possible from cuttings of the rhizome. The substrate had a significant influence on the development and survival of cuttings, either in nursery or in natural environment. Cuttings grow differently under chassis according to the different culture media used throughout this study. Results show that the sand/marshy soil substrate mixture is more efficient than the other two experimental substrates (sawdust/marshy soil and sand/sawdust/marshy soil), and is significantly closer to the results obtained in the marshy soil substrate, then the sand/sawdust/marshy soil substrate and finally sawdust/marshy soil which proved less effective during the experiment, as well as at the level of the number of leaves and appearance of shoots on the cuttings of *H. azurea*. It is also noteworthy to observe that the average number of dead cuttings was very high in the sawdust/marshy soil substrate at the end of the experiment in the nursery as compared to other substrates used. Seedlings from the sand/marshy soil mixture and marshy soil substrates also performed better in the natural environment unlike seedlings from the other mixtures (sawdust/marshy soil and sand/sawdust/marshy soil).

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