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# COMBINED DORMANCY BREAKING AND SEED GERMINATION OF *Hieronyma macrocarpa* Müll.Arg. (Phyllanthaceae)

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**Abstract:** The seeds of *Hieronyma Macrocarpa* Müll.Arg. They present a combined dormancy, which does not allow obtaining high germination percentages both at the level of natural regeneration and under controlled conditions of sexual reproduction. The objective of the research was to apply different pre-germination treatments to break dormancy in the seeds of *Hieronyma Macrocarpa* Müll. Arg., they are looking for a greater germination capacity in the shortest possible time. Regarding the methodology, the statistical design used was an AxB factorial arrangement,with four levels per factor; where, factor A were the pre-germination mechanical scarification and leaching treatments and factor B pre-germination stratification treatments; an unrestricted random design was used with 16 treatments, four repetitions for each treatment, 64 experimental units and 20 seeds per experimental unit, giving a total of 1 280 seeds used throughout the study; for the analysis of seed trees, fruits and seeds, descriptive statistics of the variables studied were used; for the germination analysis, it was determined that the data complied with the parametric assumptions of Normality (Shapiro Wilks Test; p-value ≥ 0.05) and Homoscedasticity (Levene Test; p-value ≥ 0.05). Regarding the results, the optimal pre-germination treatments for germination were those that received mechanical scarification, the best result being the mechanical scarification with sandpaper no. 80 for five minutes using a substrate composed of 80% black earth and 20% sand, obtaining a result of 82, 5 % germination, constituting the highest percentage achieved in similar studies of the species. The conclusion was that the application of pre-germination treatments breaks the combined dormancy and increases the percentage and speed of germination. A total of 5 % germination, constituting the highest percentage achieved in similar studies of the species. The conclusion was that the application of pre-germination treatments breaks the combined dormancy and increases the percentage and speed of germination. 5 % germination, constituting the highest percentage achieved in similar studies of the species. The conclusion was that the application of pre-germination treatments breaks the combined dormancy and increases the percentage and speed of germination.
**Keywords**: pre-germination treatments, capacity, speed, time

## INTRODUCTION

The genus Hieronyma consists of 26 species distributed in tropical America. In Ecuador 10 species are represented, one of them is*Hieronyma macrocarpa*Müll. Arg. known by the common name of motilón (Franco 1990, Missouri Botanical Garden 2021).

*H. macrocarpa*It is naturally distributed between Colombia, Ecuador, Peru and Venezuela. In Ecuador H. macrocarpa inhabits the evergreen montane forest in the eastern and western cordillera of the Andes, in an altitudinal range that goes from 1,100 to 3,500 m asl (Prado & Valdebenito 2000, Ministry of the Environment [MAE] 2013, Lojan 1992, Missouri Botanical Garden 2021).

*H. macrocarpa*It is a species with great potential for multiple use and multipurpose, due to the quality and quantity of timber products, non-timber forest products and environmental services it provides.

The wood is of high density, 0.72 g/cm3 (Cubillos et al. 2019) and excellent quality, it is used in local constructions, in the manufacture of furniture, formwork, pillars, floors and decorative sheets, sleepers, fence posts and logging residues are used for wood energy (Cuamacás & Tipaz 1995, Lojan 1992).

Non-timber products are obtained mainly from its fruits and leaves. Motilón fruits have purple pulp due to the presence of

anthocyanins,it has been shown that each gram of motilón contains 17.18 milligrams of anthocyanins(Santacruz et al. 2012); They also have a high content of antioxidants, vitamin C and natural pigments (Mina & Torres 2015). According to Matute (2019) the fruits of H. macrocarpa have excellent organoleptic and nutritional characteristics for the consumer, they have potential for the development of gastronomic food and beverage proposals, these are highly desired by local communities for the preparation of traditional beverages., like the purple wash.

The seeds of H. macrocarpa constitute a source of food for some species of wildlife, mainly squirrels and birds; are a fundamental element in the diet of Andean bears (Troya et al. 2004), their leaves are used as fodder for bovines, additionally the species plays a key role in the provision of environmental services such as carbon capture, contribution of organic matter to the soil and maintains habitats that allow the long-term permanence of wildlife diversity, especially birds.

The entire potential of the species is threatened by the level of fragmentation of the landscape where it is found, mainly due to the expansion of the agricultural frontier and the uncontrolled felling of the forests where it inhabits.Due to the aforementioned, the Ministry of the Environment of Ecuador considers Hieronyma macrocarpa under standard 128 within the species of conditional use (MAE 2006).

The fruit is a fleshy, oval-shaped drupe, with a length of up to 2.5 cm, its color is green and as it matures it turns purple or reddish, sweet in taste, and contains a single seed (López 2004). The seeds are globose in shape, dark brown in color with a porous and hard testa. The embryo is large in relation to the size of the seed, it is a good source of lipids (Vargas 2002).

The fruits of H. macrocarpa take between five and six months to reach physiological maturity (Alvarado & Encalada 2010).

The seed of H. macrocarpa presents combined exogenous/mechanical dormancy with endogenous/physiological-intermediate, in which the dormancy affects the seminal coat and the embryo at the same time, therefore, the viable seeds fail to complete the germination process, although the interaction between environmental factors is favorable for its development (FAO 1991).

Under natural conditions, the seeds of the species can remain dormant for long periods of time and will germinate when factors such as: water, microbial activity or are corroded by an acid in the digestive tract of an animal, have softened the seminal coat, making them permeable. to water and gas exchange. This process can take several months and even years (FAO 1991).

Ferney et al. (2001) affirm that the motilón seed needs to be subjected to pre-germination treatments that allow breaking the dormancy imposed by the cover that delays the emergence of the embryo. Iglesias (2016) indicates that the dormancy induced by the seed coat is broken by placing the seeds of H. macrocarpa in immersion in water at 100 0C. In addition to this, they suggest the use of substrates with good physical, chemical and biological characteristics in the seedling rearing stages.

Alvarado & Encalada (2010) in a study on H. macrocarpa seed germination obtained zero germinated seeds due to their latency. Benavides & Ruano (2018) applying pre-germination treatments obtained a germination percentage of less than 30 %, in the control treatment they did not register germinated seeds.

## MATERIALS AND METHODS

The seeds of H. macrocarpa were collected from relict trees and on boundaries in

the El Chamizo sector (0°32'32.49"N and 77°46"14.65"W) Montufar municipality, Carchi province. Trees were selected by non-probabilistic sampling, applying the parameters established by Heredia & Hofstede (1999), adapted by Ordoñez et al. (2001), where the individual phenotypic characteristics of the trees were evaluated, simultaneously with the methodology of (Lombardi et al. 2013) in which their dasometric characteristics were evaluated.

The ripe fruits from which the seeds were extracted for the research were fresh, taken directly from the plant. They were collected on May 10, 2021, the field trials (nursery treatments) were installed on July 6, 2021.

Seed analysis under procedures of the International Seed Testing Association (ISTA) standard was carried out in the wood and xiloteca laboratory located at the Yuyucocha Experimental Farm (00°21'53"N and 78°06'32"W), owned by the Technical University of the North, in Ibarra, Ecuador.

The evaluation of combined pre-germination treatments was carried out in the nursery located in the Huaca parish (00°38'40.4"N and 77°43'37.8"W), San Pedro de Huaca Municipality, Carchi Province. The nursery is located at an altitude of 2,900 m above sea level, with an average annual temperature of 12.0 °C, and average annual rainfall of 1,600 mm year-1. and a relative humidity of 80%.

The criteria used to define the trees to collect the seeds from were: i) diameter measured at 1.30 m from the ground; ii) total height; iii) stem shape; iv) bifurcation height; v) branch insertion angle; vi) shape of the cup; vii) crown diameter; viii) phytosanitary status, ix) phenological status, x) fruits/tree.

The seed testing protocols followed the International Seed Testing Association Standards (ISTA 2016). Tests were carried out to evaluate the number of seeds kg-1, germination capacity, purity percentage and moisture content.

The research in the nursery was established under an unrestricted random design in AxB factorial arrangement, with four levels per factor; where, factor A were the pre-germination mechanical scarification and leaching treatments and factor B pre-germination stratification treatments; with a total of 16 treatments, four repetitions each, 64 experimental units and 20 seeds per experimental unit, giving a total of 1 280 seeds used throughout the study.

Factor A levels were: A1) mechanical scarification with no. 80 sandpaper for five minutes; A2) immersion in hot water at 90-100 °C, for 10 minutes; A3) immersion in cold water for 24 hours with a change of water every 12 hours; A0) without pregerminative treatment. Factor B levels: B1) black earth (80%) + sand (20%); B2) black earth (50%) + organic fertilizer (50%); B3) black earth (70%) + rice husk (30%) and B0) black earth.

The variables used in the analysis of the germination results were analytical, being the following: germination capacity, average germination time, germination speed coefficient, germination speed index, germination index and latency time ( Table 1).

For seed analysis, we useddescriptive statistics of the variables studied; for the germination analysis, it was determined that the data complied with the parametric assumptions of Normality (Shapiro Wilks Test; p-value ≥ 0.05) and Homoscedasticity (Levene Test; p-value ≥ 0.05). The Analysis of Variance was applied to the data that met the parametric assumptions, in the cases that there were significant differences between the treatments, the Tukey test was applied with $\alpha = 0.05$, in the cases that did not meet the assumptions the Kruskal Wallis test was applied with $\alpha = 0.05$. The number of germinated seeds was counted daily for 69

| Variable | Equation | Description |
|---|---|---|
| germination capacity (%) | $PG = \frac{Sg}{Ss} * 100$ | PG=germination percentage<br>Sg=total germinated seeds.<br>Ss=total seeds sown. |
| Average germination time (days) | $TPG = \frac{\sum(n_i t_i)}{\sum n_i}$ | TPG=average time to germination<br>ti=number of days after sowing.<br>N=number of seeds germinated on day i. |
| Germination rate coefficient (days-1) | $CVG = \frac{\sum n_i}{\sum(n_i t_i)}$ | CVG=germination speed coefficient<br>ti=number of days after sowing.<br>n=number of seeds germinated on day i. |
| Germination speed index | $M = \sum\left(\frac{n_i}{t}\right)$ | M=germination speed<br>ni=number of seeds germinated on day i.<br>t=germination time from sowing to the germination of the last seed. |
| germination rate | $IG = \frac{\sum(n_i t_i)}{N}$ | GI=germination index<br>ni=number of seeds germinated on day i.<br>ti=number of days after sowing.<br>N=total seeds sown. |
| latency time | | Time required for the start of germination. Demonstrates the effect of treatments on breaking dormancy. |

Table 1: Detail of the equations of the analyzed variables.

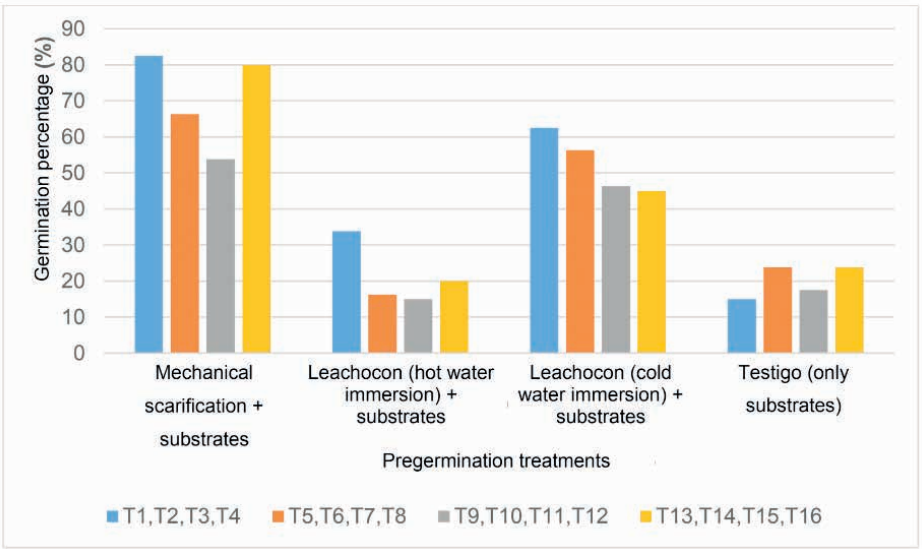


Figure 1. Percentage (%) of germination of seeds of*Hieronyma macrocarpa*.

days.

## RESULTS AND DISCUSSION

### SEED ANALYSIS

The average number of H. macrocarpa seeds was 2 383/kg; the coefficient of variation of the weight of the seeds reflected a value of 2.88 %, this value is lower than the maximum prescribed by ISTA (4 %), which demonstrated that the sample was homogeneous and it was not necessary to take new samples (Hurtado - Trejo et al. 2020). In the province of Cañar - Ecuador, López (2004) found between 1,140 and 1,700 seeds/kg.

The percentage of purity of the H. macrocarpa seeds was 96 %. Scientific research on this parameter in the species is non-existent, however studies carried out on H. asperifolia in recent years account for 42 % in an investigation carried out by Quiroz (2019).

The average moisture content of H. macrocarpa seeds was 16.14 %, a reference that is low when compared to other studies such as Quito & Yunga (2019) who obtained 53.06 %.

### GERMINATION CAPACITY

The germination capacity of H. macrocarpa seeds varied depending on the environment where the seeds germinated and the different pre-germination treatments applied.

At the laboratory level under controlled conditions of temperature (24 oC) and humidity (35 %) and without applying pre-germination treatments, the average germination percentage was 26.5 %; In the field with conditions of average temperature (12 oC) and average humidity (80 %) to the environment and without pre-germination treatments, the average germination percentage was 20.02 %.

Using pre-germination treatments, the highest germination was obtained, which was 82.5 % applying the scarification treatment with sandpaper No. 80 for 5 minutes and a culture medium made up of 80 % soil and 20 % sand, constituting the highest percentage. high achieved in similar studies of the species (Figure 1).

Ferney et al. (2001) obtained the best germination percentage, this being 30 % when studying the mechanical scarification treatment with No. 150 sandpaper in 20 minutes using sawdust substrate.

### MEAN TIME TO GERMINATION (TPG)

The average germination time at the general level of the research ranged between 102 and 129 days (Figure 2), in treatments T1 and T14 germination occurred in less time where the TPG was 102 days, a result similar to that obtained by Iglesias ( 2016); Other authors such as López (2004) indicate that this process can take up to 200 days.

The coefficient of variation reflected a value of 5.93 %, the data complies with the parametric assumptions of normality (Shapiro Wilks, p-value = 0.4586) but not with homoscedasticity. Through the analysis of non-parametric variance with the Kruskal Wallis test ($\alpha = 0.05$), it was shown that there are significant differences between the treatments studied.

### GERMINATION SPEED COEFFICIENT (CVG)

From the means test, it was determined that at least one of the studied treatments significantly influenced the behavior of the germination rate coefficient of H. macrocarpa, the control treatments presented the lowest values, while the T1 and T14 treatments presented the maximum CVG with 0.98 % (Figure 3), which allows us to infer that the applied pre-germination treatments increased the germination speed coefficient of H.

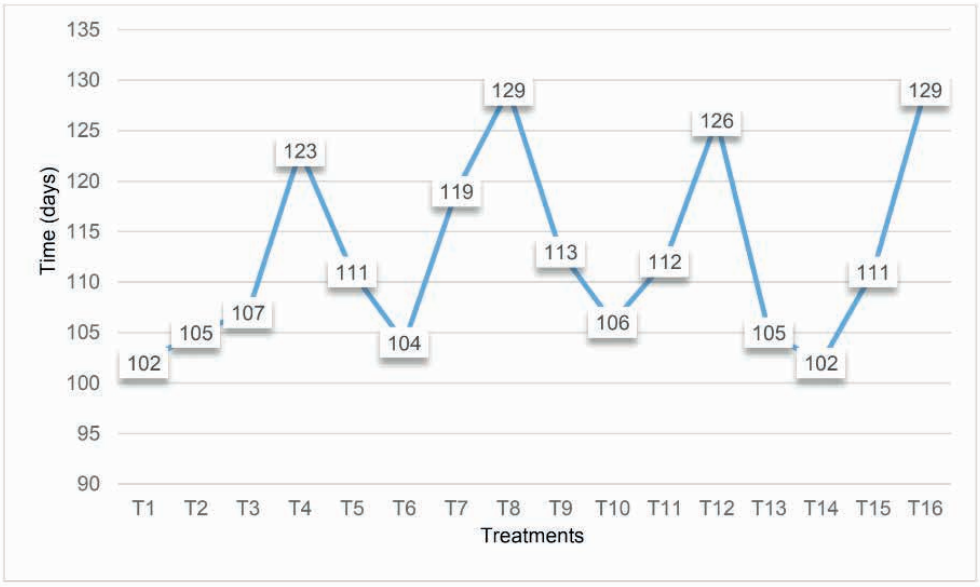


Figure 2. Average germination time of H. macrocarpa seeds

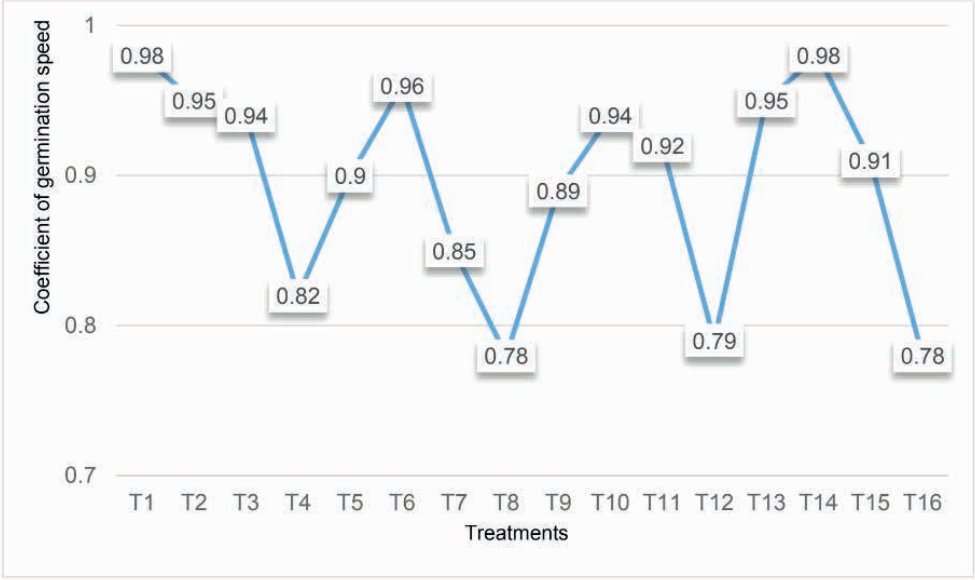


Figure 3.Seed germination rate coefficient of H. macrcocarpa.

macrocarpa seeds.

According to (Horak & Wax 1991), high values of the germination speed coefficient are an indicator of the directly proportional relationship: the higher the germination speed, the higher the germination percentage. The results of the present investigation partially agree with the authors, since, in the control treatments where the CVG is the lowest, the germination percentages are also among the lowest; On the other hand, T14, which presented a high CVG of 0.98 %, registered a lower germination percentage.

**GERMINATION INDEX (GI)**

The seeds that received the mechanical scarification treatments reached the highest germination index, with the maximum value of 84.25 achieved by T1; the leaching treatments (immersion in cold water for 24 hours) follow with 66.59 and 65.23 (Figure 4).

The coefficient of variation reflected a value of 35.95%, a data transformation to square root was performed and a coefficient of variation of 19.98% was achieved, the data complies with the parametric assumptions of normality (Shapiro Wilks, p- value = 0.7617) and homoscedasticity of variances (Levene, p-value = 0.1270), therefore the analysis of variance was carried out as shown in Table 2.

Through the ADEVA it was determined that there are highly significant differences between the treatments and the levels of factor A, therefore, the types of substrates did not influence the germination index of H. macrocarpa. Applying the Tukey test with α = 0.05, it was determined that the treatments T1 and T13 with 9.16 presented a significantly different germination index.

Hartmann & Kester (1997) indicate that scarification increases germination by allowing the entry of water into seeds that have an impermeable testa. In the present study, mechanical scarification treatments significantly increased the GI of H. macrocarpa seeds.

**GERMINATION SPEED INDEX (IVG)**

The analysis of variance reflected highly significant differences between the treatments studied and the levels of factor A. Using the Tukey test (α = 0.05), it was determined that treatment T1 with an IVG of 0.163 germinated seeds/day was the one with the highest effect.

When relating the germination speed index with the germination index, a strong correlation was found between these two quantitative variables, an example is treatment T1, which presents the highest value of the GI and also the highest value in terms of IVG (Figure 5 ).

**LATENCY TIME**

The latency time decreased after the seeds were subjected to pre-germination treatments. In the seeds that did not receive any type of treatment, be it mechanical scarification or leaching, the longest latency time was 123 days, while the seeds that were subjected to treatments such as mechanical scarification this time was 88 days (Figure 6).

The coefficient of variation reflected a value of 6.64 %, the data complies with the parametric assumptions of normality (Shapiro Wilks, p-value = 0.6121), but not with those of homoscedasticity. Through the analysis of non-parametric variance with the Kruskal Wallis test (α = 0.05), it was shown that there are significant differences between the treatments studied.

The effectiveness of pre-germination treatments to reduce latency time has been reported by other authors. Ferney et al. (2001) points out that when using the mechanical scarification treatment (No. 80 sandpaper, for 05 min) the seeds broke dormancy at 62 days. Iglesias (2016) using the immersion treatment in hot water, managed to start germination

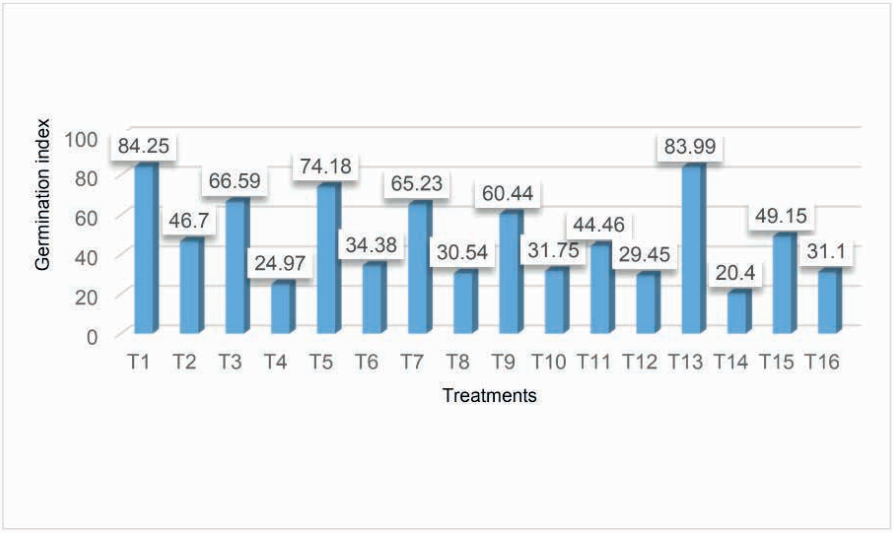


Figure 4. Germination index of H. macrocarpa seeds.

| FV | SC | gl | CM | F | p-value |
|---|---|---|---|---|---|
| Treatments | 136.15 | fifteen | 9.08 | 4.87 | <0.0001 |
| Factor A: Pre-germination treatments | 108.76 | 3 | 36.25 | 19.43 | <0.0001 |
| Factor B: Types of substrates | 6.41 | 3 | 2.14 | 1.15 | 0.3419 |
| Factor A*B | 13.52 | 9 | 1.5 | 0.81 | 0.614 |
| Mistake | 76.48 | 41 | 1.87 | | |
| Total | 212.64 | 56 | | | |

*Note:*FV: Source of Variation; SC: sum of squares; df: degrees of freedom; CM: mean square; F: Fisher calculated.

Table 2. Analysis of variance for the germination index of H. macrocarpa seeds.

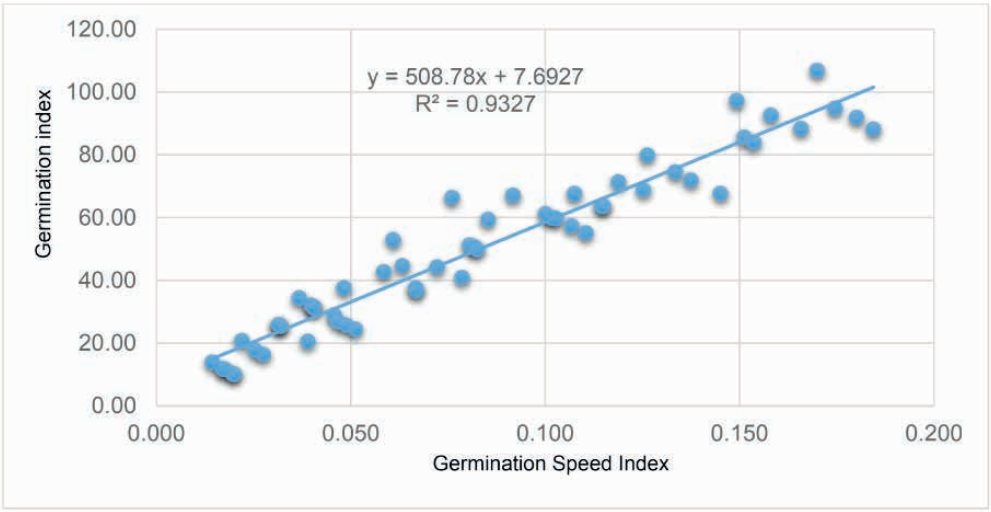


Figure 5. Germination patterns of H. macrocarpa seeds with different pre-germination treatments.

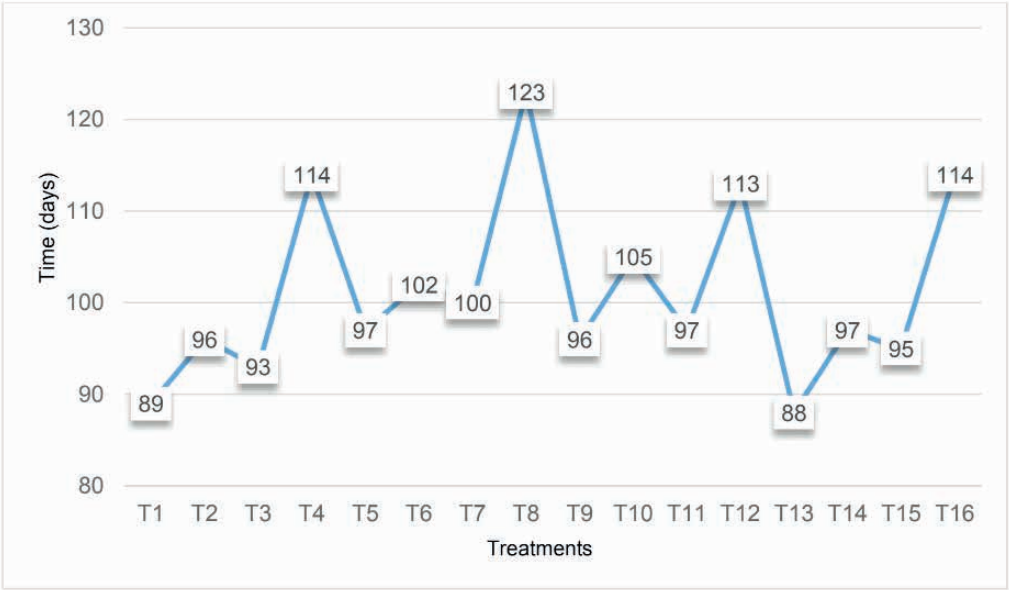


Figure 6. Latency time of H. macrocarpa seeds.

after 40 days.

## CONCLUSION

The application of pre-germination treatments breaks the combined dormancy and increases the germination of Hieronyma macrocarpa Müll.Arg seeds. (Motilón), with the particularity that the mechanical scarification treatment with sandpaper No. 80 for 5 minutes and a culture medium made up of 80% soil and 20% sand, registers the highest germination percentage so far obtained in similar studies.

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