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## Survival of *Swietenia mahagoni* in the xerophytic corridor of the Guantánamo valley

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## ABSTRACT

The survival of *Swietenia mahagoni* obtained in nursery with the application of arbuscular mycorrhizal fungi (AMF) and different organic substrates was evaluated. Plants produced on two levels of substrates: cocoa husk, coconut fiber and composted pine sawdust in 6:2:2 and 2:6:2 ratios, and four levels of mycorrhizal strains: *Glomus cubense*, *Rhizoglomus irregulare*, *Funneliformis mosseae* and non-mycorrhizal, were used for a total of eight treatments. Plants in the field were distributed under a randomized block experimental design, with four replications per treatment; the planting frame used was 2 x 2 m. The plantation was done after 30 days and the survival was assessed monthly until 12 months. During this time, the morphological variables: height and diameter were registered, and the height to diameter ratio and the relative growth rate were calculated. Survival analysis was performed using the Kaplan-Meier estimator and proportional hazards regression was performed to determine the risk of mortality as a function of morphological variables. At 12 months after planting, an average survival rate of 86.30 % was obtained, with higher percentage for plants inoculated with *Glomus cubense* and *Rhizoglomus irregulare* strains in the substrate composed of 20 % cocoa husk + 60 % coconut fiber + 20 % sawdust. Diameter was the morphological variable most related to the risk of mortality in the planting sites.

**Keywords:** Forest plantation; Reforestation; Substrate; *Arbuscular mycorrhiza*.

## RESUMEN

Se evaluó la supervivencia de *Swietenia mahagoni* obtenidas en vivero con la aplicación de hongos micorrízicos arbusculares (HMA) y diferentes sustratos orgánicos. Se utilizaron plantas producidas en dos niveles de sustratos: cascarilla de cacao, fibra de coco y aserrín de pino compostado en proporciones 6:2:2 y 2:6:2, y cuatro niveles de cepas micorrízicas: *Glomus cubense*, *Rhizoglomus irregulare*, *Funneliformis mosseae* y sin micorrizar, para un total de ocho tratamientos. Las plantas en el campo se distribuyeron bajo un diseño experimental de bloques al azar, con cuatro repeticiones por tratamiento; el marco de plantación utilizado fue de 2 x 2 m. La plantación se realizó a los 30 días y la sobrevivencia se evaluó mensualmente hasta los 12 meses. Durante este tiempo se registraron las variables morfológicas: altura y diámetro, y se calculó la relación altura/diámetro y la tasa de crecimiento relativo. El análisis de la supervivencia se realizó por el estimador de Kaplan-Meier y se realizó una regresión de riesgos proporcionales para determinar el riesgo de mortalidad en función de las variables morfológicas. A 12 meses de plantadas, se obtuvo una supervivencia promedio de 86,30 %, con mayores porcentajes para las plantas inoculadas con las cepas *Glomus cubense* y *Rhizoglomus irregulare* en el sustrato compuesto por 20 % de cascarilla de cacao + 60 % de fibra de coco + 20 % de aserrín. El diámetro fue la variable morfológica que más se relacionó con el riesgo de mortalidad en los sitios de plantación.

**Palabras clave:** Plantación forestal; Reforestación; Sustrato; *Micorriza arbuscular*.



## RESUMO

Evaluo-se a sobrevivência de *Swietenia mahagoni* obtidas em viveiro com a aplicação de fungos micorrízicos arbusculares (FMA) e diferentes substratos orgânicos. Utilizaram-se mudas produzidas em dois níveis dos substratos: casca de cacau, fibra de coco e casca de pinus em proporção 6:2:2 e 2:6:2, e quatro níveis de inóculo micorrízicos: *Glomus cubense*, *Rhizoglomus irregulare*, *Funneliformis mosseae* y sim micorrizar, para um total de oito tratamentos. A planta no campo foi distribuída ao acaso debaixo de um designio experimental de blocos, com quatro repetições por tratamento; o quadro de plantio utilizado foi de 2 x 2 m. Depois de 30 días da implantação do experimento no campo, cada mes evaluó-se a sobrevivência hasta os 12 meses. Ao mesmo tempo, se registraram a variável altura das plantas e diâmetro do colo, com as cuais calculo-se o cociente entre a altura e diâmetro e a Tasa Relativa do Crecimento. O analeses da brevivência foi por o método Kaplan-Meier e realizo-se uma regreção do risco proporcionais para determinar o riesco da mortalidade. A 12 meses de plantadas, obtuvo-se uma sobrevivência promedio de 86,30 %, com mior representatividade para as plantas inoculadas com os inóculos *Glomus cubense* e *Rhizoglomus irregulare* no substrato contituído por 20 % de casca de cacau + 60 % de fibra de coco + 20 % casca de pinus. O diâmetro foi a variável morfológica que mais se relaciono com o risco da mortalidade nos sitios de plantação.

**Palavras-chave:** Plantação florestal; Reforestação; Substrato; *Micorriza arbuscular*.

## INTRODUCTION

In recent years, the use of organic substrates and AMF constitute feasible alternatives for plant nutrition (Valkinir *et al.*, 2017; Brundrett and Tedersoo 2018; Valenzuela 2019), fundamentally in arid and semi-arid environments, as they help organisms to overcome environmental stress conditions (Martínez-García 2011).

These alternatives can be applied in areas where reforestation works must be more intentional due to the edaphoclimatic characteristics of the area, as is the case of the Fincas Forestales Integrales de la localidad de Paraguay, located in the semi-arid zone of the Guantánamo Valley with conditions of prolonged droughts, high temperatures, high salinity and low fertility, factors that can become limiting in the growth and survival of existing forest plantations (Villamet 2018; O'Farrill *et al.*, 2018).

The reforestation programs carried out in these farms have allowed the recovery of the topsoil and soil improvement, as well as the establishment of 40.6 hectares of new plantations (Villamet 2018). However, there are empty spaces or gaps of light within the plantations that can be corrected using the enrichment technique as a function of increasing biodiversity (Alvarez 2017).

*Swietenia mahagoni* L. Jacq. is one of the species selected for irregular enrichment, considering that it boasts characteristics such as: autochthonous, facultative heliophilous, stabilizing, colonizing different successional stages, tolerant to competition and relatively high pH, as well as recognized mycorrhizal dependence (Abd El-Kader *et al.*, 2016 and Ricardo *et al.*, 2016).



The objective of this study was to evaluate the survival and growth of *Swietenia mahagoni* obtained in nursery with the application of arbuscular mycorrhizal fungi (AMF) and different organic substrates in the xerophytic corridor of southern Guantánamo province.

## MATERIALS AND METHODS

### Characteristics of the study area

The work was carried out in areas of the Finca Forestal Integral "La Acacia", belonging to the Empresa Agroforestal Guantánamo, located at the geographical coordinates 20°06'05,86" North latitude and 75°08'52,20" West longitude at 23 m a.s.l. The site is located in the middle of a transition forest from semi-arid to temperate forest. The predominant vegetation is composed of *Casuarina equisetifolia* Forst. and *Caesalpineae violaceae* Mill. (Standl.), and in greater quantity *Lysiloma latisiliqua* (L.) Benth and *Leucaena leucocephala* L. The specific area is characterized as an open space, free of vegetation and previously used for cattle grazing. The terrain has zenithal exposure.

The region has an average temperature of 26.27°C, an absolute maximum of 32°C and an average absolute minimum of 20°C, while the average annual rainfall is 851.1 mm, exceeding 100 mm per month only in May, September and October, while the rest of the months represent dry periods, where potential evaporation is greater than the sum of precipitation (INSMET 2021).

In general, it is characterized by a very dry climate, with predominantly high temperatures and low rainfall. The soil is Alluvial (Fluvisol-agrogenic-carbonate) according to the methodology of Hernández et al., (2015), with pH of 7.3, low organic matter content (2.2 %) and assimilable phosphorus content (2.0 mg 100 g<sup>-1</sup>).

### Plant production

The plant material was produced in a nursery located at the Centro de Estudio de Tecnologías Agropecuarias y Forestales (CETAF), located 6 km from the city of Guantánamo at 87 m altitude (INSMET 2021). *Swietenia mahagoni* seeds were obtained from mature fruits of a seed mass belonging to Empresa Agroforestal Guantánamo. The seedlings were produced in 200 cm plastic tubes<sup>3</sup> with substrates composed of cocoa husk, coconut fiber and sawdust of *Pinus cubensis* decomposed in a 6:2:2 and 2:6:2 ratio, which were inoculated with the strains: *Funneliformis mosseae*, *Glomus cubense* and *Rhizoglyphus intraradices*, with 30, 33 and 36 spores g<sup>-1</sup> of inoculant, respectively, coming from the strain of the National Institute of Agricultural Sciences (INCA). In all inoculated substrates, seedlings presented mycorrhization percentages between 40.75 and 52.45 % (Falcón et al., 2021).

### Land preparation and plantation establishment

The site was prepared manually by clearing the soil with a machete, eliminating diseased trees, poorly formed trees, shrubs and weeds, as part of the silvicultural interventions, according to the needs of the plantation (Álvarez and Varona 2006). In the first month after transplanting, weekly irrigations were carried out, with approximately 1 L of water per plant, using a watering can. Re-inoculation was carried out at the time of



transplanting with the following strains: *Funneliformis mosseae*, *Glomus cubense* and *Rhizogloium intraradices*, with 30, 33 and 36 spores g<sup>-1</sup> of inoculant respectively, at a rate of 10 g per plant located under the roots and in direct contact with them.

Three natural clearings of different dimensions were identified in the study area (325, 328 and 346 m<sup>2</sup>), the size was estimated as the area of an ellipse; the main axes were measured from north to south and from east to west with a tape measure. The enrichment experiment was carried out using a randomized block design, with three replications, at a spacing of 2 x 2 m, where 30 plants per treatment were used for a total of 270 individuals, with a bifactorial arrangement (2x4). The factors consisted of two levels of substrates (cocoa husk, coconut fiber and pine sawdust in proportions 6:2:2 and 2:6:2) and four levels of mycorrhizal strains (*Glomus cubense*, *Rhizogloium irregulare*, *Funneliformis mosseae* and non-mycorrhizal), for a total of eight treatments (Table 1).

**Table 1.** - Description of treatments

Treatment	HMA strains	Substrate	Substrate composition (%)
<b>T1</b>	-	S1	60Cc + 20Fc + 20As
<b>T2</b>	-	S2	20Cc + 60Fc + 20As
<b>T3</b>	<i>Glomus cubense</i>	S1	60Cc + 20Fc + 20As
<b>T4</b>	<i>Glomus cubense</i>	S2	20Cc + 60Fc + 20As
<b>T5</b>	<i>Rhizogloium irregulare</i>	S1	60Cc + 20Fc + 20As
<b>T6</b>	<i>Rhizogloium irregulare</i>	S2	20Cc + 60Fc + 20As
<b>T7</b>	<i>Funneliformis mosseae</i>	S1	60Cc + 20Fc + 20As
<b>T8</b>	<i>Funneliformis mosseae</i>	S2	20Cc + 60Fc + 20As

Cc: cocoa husk; Fc: coconut fiber; As: sawdust; AMF: arbuscular mycorrhizal fungi.

A total of 270 plants of each treatment were planted in the field, after the last sampling in the nursery (120 days), to be evaluated over a period of 12 months, according to the edaphoclimatic characteristics of the region. Planting with the *S. mahagoni* species was done with irregular enrichment (Álvarez 2017) and under the silvicultural treatment that is carried out annually in the Integral Forest Farms, according to the Development Plan of the Guantánamo Agroforestry Enterprise until 2030 (MINAG 2021).

### Variables evaluated

At the time of planting, initial morphological variables were measured to include them as covariates and analyze their effect on the survival of each treatment, the variables considered were: plant height (cm), root collar diameter (mm) and slenderness (H/D). After planting, survival was evaluated every month up to 12 months. For each measurement, values of 0 or 1 were assigned for dead and live plants, respectively.



Growth in height (measured from the base of the stem to the main apex) and diameter (measured at the base of the stem), was analyzed as relative growth rate (RGR) with the following equation (Cregg 1994) (Equation 1).

$$TRC = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1} \quad (1)$$

Where:  $CRR$  = Relative Growth Rate in diameter ( $\text{mm mm}^{-1}$ ) or height ( $\text{cm cm cm}^{-1}$ ) in 12 months;  $X_2$  = value of the response variable at the end of the evaluation period and  $X_1$  = initial value of the variable, at the moment of establishing the plantation;  $t_1$  = date of planting and  $t_2$  = date of final evaluation, the difference was in months.

### Statistical analysis

The analyses of variance were performed for growth variables (height, diameter and relative growth rate) since the data fit the a normal distribution, when significant statistical differences existed, the Duncan's Multiple Range Test ( $p \leq 0.05$ ) was applied.

Differences in survival between treatments were evaluated by the non-parametric LogRank test, based on survival curves constructed by the Kaplan-Meier estimator (Kaplan y Meier 1958) for which the survival function is defined as (Equation 2).

$$S(t) = P(T \geq t) \quad (2)$$

Where:  $S(t)$  is the probability of a death occurring in at least  $T$  time, as large as time  $t$ . For this analysis, the status of each plant (alive or dead) at the end of the evaluation period is considered, as well as the lifetime of the plant.

A second analysis was a Cox proportional hazards regression, which allowed estimating the effect of mycorrhization considering morphological variables (covariates), which change over time. In a proportional hazards model, the hazard of an individual  $i$  at time  $t$ , or  $h_i(t)$ , is the product of the hazard function ( $h_0$ ) of unspecified reference and an exponential function of  $k$  covariates (Equation 3).

$$h_i(t) = h_0(t)e^{(\beta_1 t_{i1} + \dots + \beta_k t_{ik})} \quad (3)$$

The nonparametric Cox model estimates a coefficient  $\beta$  for each factor or covariate in the model and tests the null hypothesis that  $\beta = 0$  using the  $X^2$  statistic. Such a coefficient explains the effect of a factor or covariate on the hazard function, that is, if the coefficient  $\hat{\alpha}$  is negative it means that the risk of death is reduced with increasing covariate, whereas a positive  $\beta$  coefficient indicates the opposite (Williams 2008). The data were analyzed with the SPSS 23.0 program for Windows.





## RESULTS AND DISCUSSION

### Survival

Twelve months after planting, *S. mahagoni* plants inoculated with the different mycorrhizal strains showed higher survival compared to those in the non-mycorrhizal treatments (T1 and T2), with the highest values for plants grown with strains *G. cubense* and *R. irregulare* in the S2 substrate composed of 20 % coconut husk + 60 % coconut fiber + 20 % sawdust (T4 and T6), with significant differences with respect to the other treatments (Table 2). Similar results have been found for this same species in plantations established in areas of the Empresa Agroforestal Baracoa, with better results for plants mycorrhized with the *Glomus cubense* strain (Falcón *et al.*, 2018).

**Table 2.** - Estimated survival by treatment according to the Kaplan-Meier method

	Treatments	Months elapsed to present a survival of less than:			Final survival (%) *
		75 %	50 %	25 %	
<b>T1</b>	S1+ No mycorrhiza	5	-	-	65,80 <sup>c</sup>
<b>T2</b>	S2 + No mycorrhiza	5	-	-	59,20 <sup>c</sup>
<b>T3</b>	S1 + <i>G. cubense</i>	-	-	-	88,30 <sup>b</sup>
<b>T4</b>	S2 + <i>G. cubense</i>	-	-	-	95, 80 <sup>a</sup>
<b>T5</b>	S1 + <i>R. irregulare</i>	-	-	-	87,50 <sup>b</sup>
<b>T6</b>	S2 + <i>R. irregulare</i>	-	-	-	95,00 <sup>a</sup>
<b>T7</b>	S1 + <i>F. mosseae</i>	-	-	-	86,70 <sup>b</sup>
<b>T8</b>	S2 + <i>F. mosseae</i>	-	-	-	88,30 <sup>b</sup>
	General	-	-	-	<b>86,30</b>

\*Unequal letters indicate significant statistical differences using the *Log-Rank* test.

Table 2 also shows mortality with an accumulated 75 % from the fifth month (January/2020), which worsened in the seventh month (March/2020) above 50 % in the non-mycorrhizal treatments (T1 and T2), but not in the mycorrhizal treatments where survival was above 75 % during the evaluation period. Mortality in the non-mycorrhizal treatments could be the result of the non-use of mycorrhizae, which can facilitate plant development in soils with low nutrient availability, in addition to facilitating water absorption through the formation of soil aggregates, creating a porous and permeable soil (Salcido-Ruiz *et al.*, 2021).

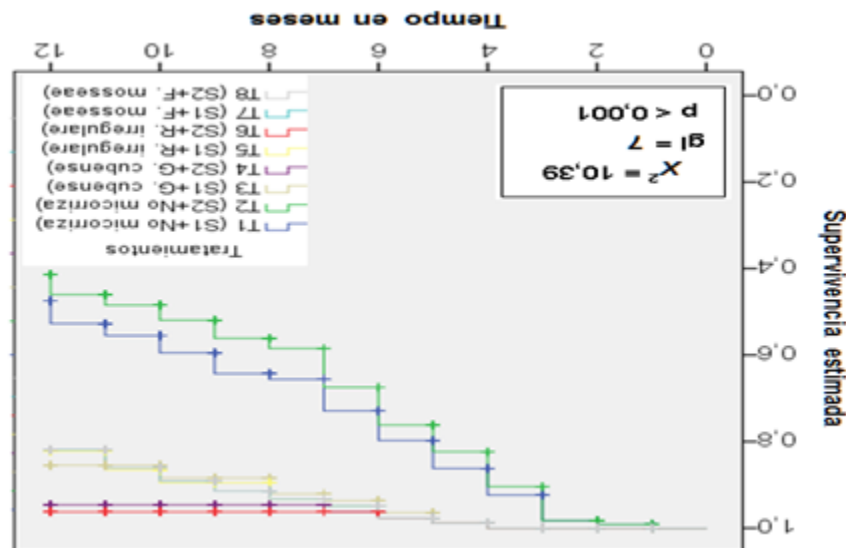




These results are in agreement with the studies of Rodríguez *et al.*, (2011) when they state that mycorrhizae are one of the most important strategies that plants have developed to survive in nutrient-poor soil conditions.

On the other hand, survival in a certain site is also affected by the physical-chemical properties of the soil such as: humidity, temperature, pH, electrical conductivity and nutrient content (Prieto *et al.*, 2018). However, in this experiment, the planting site is the same; therefore, the differences in seedling survival could have been the result of the non-use of mycorrhiza as a biofertilizer that allows plant establishment under extreme conditions such as low fertility, drought and salinity (Cardona *et al.*, 2016). Depending on the substrate used, the mycorrhizal strains used favored plant survival and performance in the field (Figure 1), since the application of AMF from the nursery production stage allows plants to maximize the availability of nutrients under limiting soil conditions (Falcón *et al.*, 2021), in addition to making better use of moisture in stressful situations such as drought, reducing water loss, as well as withstanding the pressure of competition with other plants, predation and the effect of some pathogens (Aguirre-Medina *et al.*, 2019).

Similar results have been obtained for different forest species, with better records in plants inoculated with arbuscular mycorrhizal strains (Chaiyasen *et al.*, 2017; Báez-Pérez *et al.*, 2017; Falcón *et al.*, 2018). The favorable response of mycorrhizal application is explained by the fact that the extensive mycorrhizal colonization in roots and extraradical mycelium of these species favor the formation of aggregates and soil structure in low quality sites, which can increase the suitability of the host plant in habitats with limiting resources (Martínez-García, 2011); this is relevant in sites with scarce rainfall and poor edaphic conditions, such as those that characterized the study area (Figure 1).



**Figure 1.** - Survival according to Kaplan-Meier estimator by treatments of *S. mahagoni* plantation



The significant differences between plants whose substrate was inoculated and uninoculated suggest that mycorrhizal fungi improve plant nutrition and compete with pathogens for colonization and infection sites, and can induce anatomical and morphological changes in the roots, in the populations of rhizosphere microorganisms and in plant defense mechanisms (Salcido-Ruiz *et al.*, 2021), which contribute to a higher survival rate.

The results were similar with those reported by Rodríguez (2011), who emphasizes that it is important to develop technology that allows the application of Arbuscular Mycorrhizal Fungi (AMF) in the regeneration processes of natural ecosystems and the establishment of commercial plantations, particularly in tropical regions, in order to increase the survival, quality and growth of plants in the field. Similar results have been found for *Cedrela odorata* in Veracruz, Mexico, with better results in plants inoculated with the *Rhizophagus intraradices* strain compared to noninoculated plants (Oros-Ortega *et al.*, 2015). The favorable response of inoculation with arbuscular mycorrhiza can be explained by the fact that it favors the establishment and performance of plants in the field; being inoculated allows them to take better advantage of soil moisture (Aguirre-Medina *et al.*, 2019), especially in regions with low rainfall and poor edaphic conditions such as those found in the study area.

Other results obtained by Báez-Pérez *et al.* (2017), indicate that, in severely degraded sites, the establishment of *Fraxinus uhdei* is possible, due to the effect of multiple inoculation on plants, which causes some performance variables to improve as a consequence of the interaction, as was the case of higher survival with dual inoculation with *Pisolithus tinctorius* and *Glomus intraradices*.

### Risk analysis

The Cox proportional hazards model was significant for the data set analyzed ( $X^2 = 150.32$ ,  $p > 0.0001$ ), so the overall null hypothesis that  $\beta = 0$  was rejected (Table 3).

**Table 3.** - Proportional hazards regression results

Parameters	GL	Estimator $\beta$	SE	X <sup>2</sup>	Pr < Chi <sup>2</sup>	Risk ratio
<b>Substrate</b>	1	-0,173	0,334	0,236	0,627	0,841
<b>Mycorrhization</b>		0,413	0,145	8.109	<b>0,004</b>	1,512
<b>Height</b>	1	0,383	0,057	4,182	<b>0,041</b>	1,082
<b>Diameter</b>	1	-1,073	0,196	18,088	<b>&lt; 0,0001</b>	0,342
<b>Slenderness</b>	1	3,156	0,411	0,558	0,445	2,948

The results show that, among the factors analyzed, mycorrhiza application presented a significant effect on the risk function with a positive estimator (0.413) in the comparison between treatments (mycorrhizal and non-mycorrhizal), in other words, establishing a non-mycorrhizal plant in the site conditions of the study area will have a higher risk of death compared to one established under mycorrhizal inoculation in the same site condition.



On the other hand, the analysis showed a highly significant effect of the covariate root collar diameter, with negative sign in the estimator and a hazard ratio of 0.342, which means that a 1 mm increase in plant diameter reduces the risk of death by up to 68.4 % (es decir,  $100 (1 - e^{-1,073})$ ), provided that the other variables were held constant.

The results obtained agree with [Sigala et al. \(2015\)](#), who explains that seedlings with smaller diameters may have a poor performance in the field, compared to those with larger diameters, because diameter is directly related to non-structural carbohydrate reserves ([Prieto et al., 2018](#)), and to root development ([Falcón et al., 2021](#)).

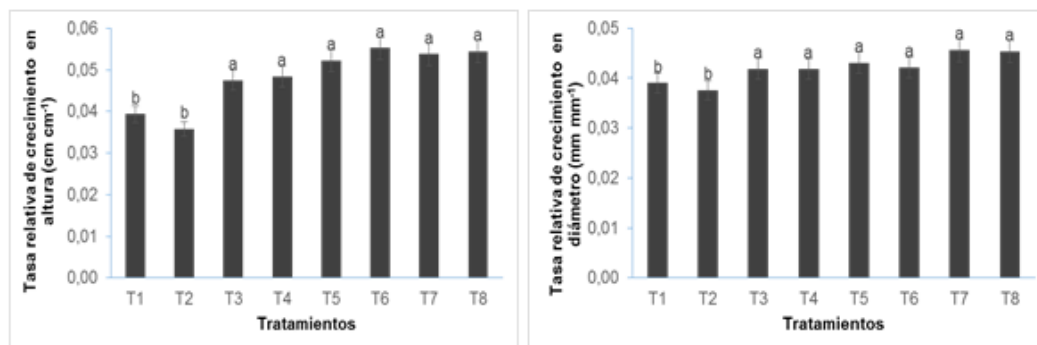
Another variable that significantly influenced survival was stem height, which, contrary to the effect of diameter, presented an estimator with a positive sign, although with a low risk ratio (1.082), indicating that an increase of 1 cm in height would increase the risk of death by 8.2 % during the first months after planting.

In studies with *Pinus pseudostrobus* species, [Sigala et al., \(2015\)](#) demonstrated that altitude negatively influenced survival during the first months of establishment, specifically, in the municipality of Galeana, Nuevo León, Mexico.

### Relative growth rate

The Relative Growth Rate in both height and diameter (Figure 2) were higher in the treatments where mycorrhiza was applied, differing statistically with the rest of the treatments, which evidences the importance of this biofertilizer in the growth in the field; these results coincide with those obtained by [Chaiyasen et al., \(2017\)](#) for *Tectona grandis* plantations, who found greater growth and survival in early stages of its development when associated with AMF, so it should be considered if the aim is to carry out restoration and reforestation activities in xerophytic forests.

Similar responses were achieved by [Falcón et al. \(2020\)](#), who pointed out that the fungus provides plants with multiple functions, among which the improvement of the absorbing surface of the root system, through a significant increase in the same, with tolerance to adverse conditions.



**Figure 2** - Relative growth rate in height and diameter of *S. mahagoni* 12 months after planting under different treatments



The non-significant increases in the response variables of the group of non-inoculated plants are due to the fact that the soil of the study area is Fluvisol, with very low phosphorus concentration. Unlike mycorrhizal plants, this factor was advantageous since they indicate that the activity and benefit of the symbiosis is more visible when they are found in phosphorus-deficient soils (Brito *et al.*, 2017); in this condition, plants inoculated with mycorrhiza show higher growth increments (Falcón *et al.*, 2021).

These results are in correspondence with Uc-Ku *et al.* (2019), who report that the fungus provides mineral nutrients, especially those that are not very mobile, such as phosphorus, zinc, copper and ammonium, absorbed from the soil solution by means of the hyphae. The mycorrhizal plant has an advantage over non-mycorrhizal plants because the external mycelium of the fungus extends further than the root hairs. In addition, fungi impart other benefits to the plant such as: improve soil aggregation, increase photosynthesis, increase soil microbiological activity, enhance nitrogen fixation by symbiotic bacteria, provide greater resistance to pests and environmental stress, stimulate the activity of growth regulating substances, making the plant drought tolerant (Piliarová *et al.*, 2019).

## CONCLUSIONS

The highest percentage of survival was shown in the S2 substrate composed of 60 % coconut fiber + 20 % cocoa husk + 20 % pine sawdust and the mycorrhizal strains *Glomus cubense* and *Rhizoglomus intraradices* (T4 and T6) with more than 90 %.

The diameter of *Swietenia mahagoni* plants is the morphological variable that best correlates with the risk of mortality. The species achieve significantly higher growth with the different mycorrhizal strains regardless of the substrate used.

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**Conflict of interests:**

The authors declare not to have any interest conflicts.

**Authors' contribution:**

The authors have participated in the writing of the work and analysis of the documents.



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