



DOI: 10.29298/rmcf.v13i74.1272

Article

Evaluation of substrates and treatments for water stress mitigation in an *Enterolobium cyclocarpum* (Jacq.) Griseb. Plantation

Evaluación de sustratos y tratamientos para mitigar el estrés hídrico en una plantación de *Enterolobium cyclocarpum* (Jacq.) Griseb.

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Fecha de recepción/Reception date: 5 de marzo de 2022

Fecha de aceptación/Acceptance date: 29 de septiembre de 2022

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Abstract

Enterolobium cyclocarpum is a multipurpose tree species whose growth and survival in plantations are unknown. The objective was to evaluate the effect of different nursery growing media and auxiliary treatments to mitigate water stress on the performance of *E. cyclocarpum* plants established in the field. The seeds were sown in three different types of substrates: 1) base mixture (peat, vermiculite and agrolite), 2) aerobic compost and 3) native soil. Each group of plants by type of substrate was divided into four subgroups that were assigned different auxiliary treatment to mitigate water stress: 1) irrigation, 2) hydrogel (2 g plant⁻¹), 3) hydrogel (4 g plant⁻¹) and 4) natural conditions (control). The results indicate that the type of substrate influences the survival and growth of the plants, being native soil the one that promotes higher values. It was found that there is a positive relationship between the diameter of the plants and the probability of survival, this variable being a basic quality indicator of the plants grown in the nursery. The auxiliary treatment of hydric stress significantly affected the growth in diameter and height, being irrigation the one that increased the values, with the exception of the diameter in the base mixture.

Keywords: Growth, combination, compost, hydrogel, survival, substrates.

Resumen

Enterolobium cyclocarpum es una especie arbórea multipropósito cuyo crecimiento y supervivencia en plantaciones se desconoce. El objetivo fue evaluar el efecto de tres medios de crecimiento en vivero y tratamientos auxiliares para mitigar el estrés hídrico en el desempeño de plantas de *E. cyclocarpum* establecidas en campo. Las semillas se sembraron en tres diferentes tipos de sustrato: 1) mezcla base (turba, vermiculita y agrolita), 2) composta aeróbica, y 3) tierra de monte. Cada grupo de plantas por tipo de sustrato se dividió en cuatro subgrupos a los que se les asignó diferente tratamiento auxiliar para mitigar el estrés hídrico: 1) riego, 2) hidrogel (2 g planta⁻¹), 3) hidrogel (4 g planta⁻¹), y 4) condiciones naturales (testigo). Los resultados indican que el tipo de sustrato influye en la supervivencia y crecimiento de las plantas, siendo la tierra de monte la que promueve mayores valores. La relación entre el diámetro de plantas con la probabilidad de supervivencia fue

positiva; esta variable es un indicador de la supervivencia en campo durante los primeros años de la plantación. El tratamiento auxiliar para mitigar el estrés hídrico afectó significativamente el crecimiento en diámetro y altura; el riego aumentó los valores, con excepción del diámetro en mezcla base.

Palabras clave: Crecimiento, combinación, composta, hidrogel, supervivencia, sustrato.

Introduction

Enterolobium cyclocarpum (Jacq.) Griseb is a multipurpose tree that grows in tropical ecosystems such as high evergreen, medium sub-deciduous, medium sub-evergreen, and low deciduous rainforests (Salas-Morales *et al.*, 2003; Pennington and Sarukhán, 2005). Its distribution area in Mexico extends from *Sinaloa* to *Chiapas* along the Pacific coast and from *Tamaulipas* to the *Yucatán* Peninsula along the Gulf of Mexico (Pennington and Sarukhán, 2005).

In its areas of distribution, this species is used mainly for the restoration of degraded sites, in agroforestry systems, and in commercial forest plantations (Muñoz-Flores *et al.*, 2016; Velasco-García *et al.*, 2019). However, survival rates are low during the first year of establishment, being associated mainly with the origin of the germplasm (Muñoz *et al.*, 2013), poor quality of the planting site, damage due to pests, diseases, or herbivory (Cibrián, 2013; Velasco-García *et al.*, 2019) and even droughts (Hernández-Hernández *et al.*, 2019).

The exploitation of *E. cyclocarpum* for timber purposes has a negative impact on its natural regeneration and, consequently, increases the fragmentation of its populations (Olivares-Pérez *et al.*, 2011). For this reason, it is essential to establish plantations that will allow its sustainable use. However, there are still gaps in knowledge regarding the best practices to increase plantation survival rates and productivity (Basave *et al.*, 2014; Hernández-Hernández *et al.*, 2019).

Nursery cultivation practices have a direct effect on plant quality and, therefore, on plantation performance (Grossnickle, 2012). Likewise, the survival and growth of plants in field depends, to a certain extent, on the methods or techniques used in the planting process (Löf *et al.*, 2012). Within this context, the objective of the present study was to evaluate the effect of different nursery growth media and auxiliary treatments to mitigate water stress on the performance of *E. cyclocarpum* plants established in field. The hypothesis was that the type of substrate influences plant survival and growth, and that auxiliary treatments may promote a better performance of this species in forest plantations or reforestations.

Materials and Methods

Seed collection and plant production

The production of the plant was carried out in the nursery of the *Los Tullillos* ranch, in the municipality *Tzitzio, Michoacán* (Figure 1). The seeds were collected in the same locality between May and June 2019. Prior to sowing, the seeds were scarified with mechanical tweezers to homogenize germination, and subsequently sown in low-density, caliber 400, 13×25 cm polyethylene bags with four perforations. The bags were filled with three different types of substrate: 1) base mix —peat, vermiculite and agrolite— at a ratio of 2:1:1, with 4 g L⁻¹ of controlled release fertilizer; 2) aerobic compost, consisting of cattle manure, and 3) forest land, obtained in an area of low deciduous forest. A total of 200 plants were produced per

substrate type. Irrigation was applied manually with a watering can every other day. The cultivation period in the nursery was three months, before the plants were taken to the field.

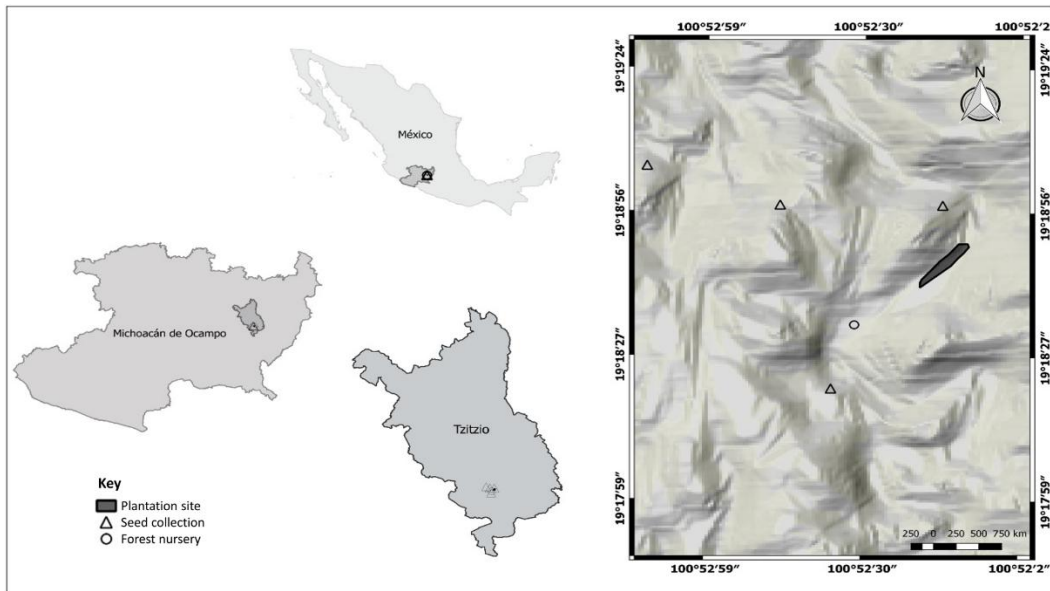


Figure 1. Location of the planting site and location of *Enterolobium cyclocarpum* (Jacq.) Griseb. trees.

Plantation establishment and monitoring

The plantation was established in 2019 in the region known as *Tierra Caliente*, at coordinates 19°18'33.5" N and 100°52'32.3" W at 750 meters above sea level, in the municipality *Tzitzio, Michoacán* (Figure 1). The predominant climate, according to Köppen's classification as modified by García (2004), is warm sub-humid A(wo)

with summer rains, mean annual precipitation of 994.3 mm and mean annual temperature of 23.4 °C (73.4 °F) (Inegi, 2017). The soil type according to the FAO/Unesco classification modified by Detenal (1979) is chromic vertisol, considered as a clayey soil of a brown or reddish hue.

The planting was carried out in the rainy season, between July 29 and August 3, 2019, on a degraded site with a history of agricultural use. 200 plants were planted per type of substrate, with real frame distribution and 4 m spacing between plants. Each group of plants by substrate type was divided into four subgroups, each of which was assigned a different auxiliary treatment to mitigate water stress: 1) irrigation, 2) hydrogel (2 g plant⁻¹), 3) hydrogel (4 g plant⁻¹), and 4) natural conditions (control). Irrigation was carried out every third day, applying 16 L per plant. The hydrogel used is a superabsorbent polymer made from potassium polyacrylate, formula $-\text{CH}_2\text{CH}(\text{CO}_2\text{K})$, with a density of 1.22 g per cm³ and an absorption capacity of 100 times its weight. This product was mixed with the substrate of the same strain and deposited at the time of planting.

The experiment was established under a split-plot design, with a 4×3 factorial arrangement of the treatments. The larger plots were assigned the treatments to mitigate water stress. The substrate type treatments (small plots) were distributed within the large plots. For each type of substrate within the large plots, there were five replicates of 10 plants, distributed completely at random. A total of 600 individuals were planted on an area of 0.96 ha. Once these were planted, the height (cm) and diameter at ground level (mm) of each plant were recorded, and a label with an identification number was placed for monitoring purposes. Ten months after establishment, plant survival was evaluated by assigning a value of 0 to dead plants and 1 to live plants. In addition, stem height (cm) was measured with a 3-meter Truper Gripper model FH-3M flexometer and diameter at ground level (mm) with a Vernier Ultratech model H-7352 were measured for each living plant.

Data analysis

The data were analyzed with the R software (R Core Team, 2020). For survival, a generalized linear model with a binomial distribution and 'logit' link function was used, in which the effect of the substrate, the auxiliary treatment, and the interaction of the two were included as explanatory variables. Initial diameter and height were also considered as covariates (Equation 1).

$$P = 1/(1 + e^{-(\alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n)}) \quad (1)$$

Where:

P = Probability of survival

α = Intercept

$\beta_1, \beta_2, \dots, \beta_n$ = Estimators associated with the explanatory variables X_1, X_2, \dots, X_n

The effect of substrate, the auxiliary treatment and their interaction on diameter and height was analyzed by means of a two-way analysis of variance (ANOVA). For the height and diameter models, the initial height and diameter were included as covariates to account for possible initial differences between treatments (Equation 2).

$$Y_{ijk} = \mu + X_k + A_i + B_j + AB_{ij} + \varepsilon_{ijk} \quad (2)$$

Where:

Y_{ijk} = Response (height or diameter) obtained for the i^{th} level of substrate and the j^{th} level of auxiliary treatment in the k^{th} plant

μ = Overall average effect

A_i = Effect attributed to the i^{th} level of substrate

B_j = Effect attributed to the j^{th} level of the auxiliary treatment

AB_{ij} = Interaction of the two factors

X_k = Effect of the initial value of height or diameter on the k^{th} plant

ε_{ijk} = Random error

In all cases, the assumptions of normality of model residuals and homogeneity of variances were checked using the Shapiro-Wilk and Levene's tests, respectively. For both variables, the transformation with the natural logarithm function was necessary to meet these assumptions ($p > 0.05$).

Results

Ten months after planting, the substrate type had a significant effect on plant survival ($Chi^2=10.62$, $p=0.005$). Plants grown on native soil showed higher survival than those grown with the base mix and compost. At the end of the study, plants grown on topsoil achieved 76 % survival, while plants with base mix and compost had 62 and 65 %, respectively. Likewise, regardless of treatment, the relationship between the survival rate and the initial diameter of individuals was significant ($Chi^2=20.51$, $p<0.001$) (Figure 2). The survival probability ratio as a function of diameter was 1.44, indicating that a 1 mm increase in the diameter of nursery plants increases the probability of survival in the field by 1.4 times. On the other hand, the auxiliary treatment to mitigate water stress did not significantly influence survival ($Chi^2=1.07$, $p=0.785$).

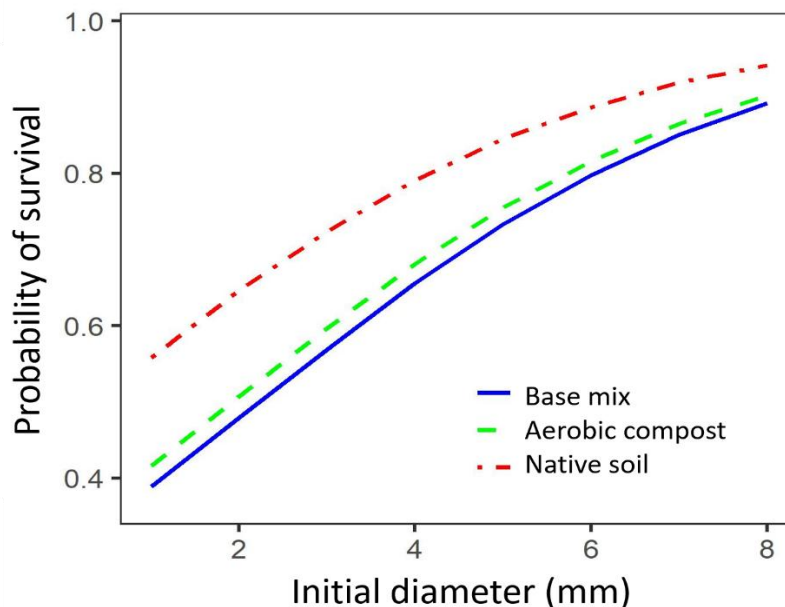


Figure 2. Probability of survival of *Enterolobium cyclocarpum* (Jacq.) Griseb. plants ten months after establishment as a function of the initial diameter at planting and the nursery growing substrate.

In general, the auxiliary treatment for mitigating water stress affected diameter growth significantly ($F=15.17$, $p<0.001$), unlike the type of substrate ($F=2.01$, $p=0.135$). However, the interaction of the auxiliary treatment with the substrate was significant ($F=4.23$, $p<0.001$). This interaction showed that the response to the auxiliary treatment was different for each type of substrate. Although irrigation increased diameter growth with respect to the control, its effect was significant only in plants grown with compost (Figure 3). The hydrogel treatments induced greater growth in diameter, with respect to the control, but only in those cultivated on native soil. Finally, the effect of the auxiliary treatments for mitigating water stress was not significant in plants grown on the base mixture, with respect to the control.

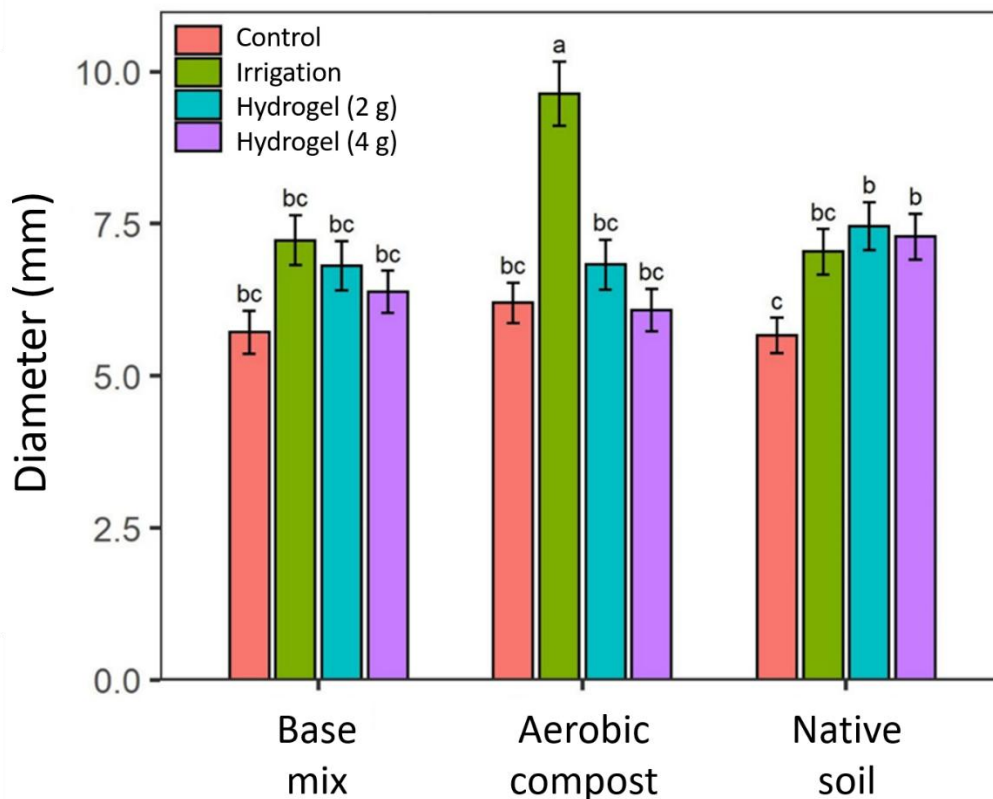


Figure 3. Mean diameters \pm SE in *Enterolobium cyclocarpum* (Jacq.) Griseb. plants ten months after establishment, grown in three types of substrates and planted with different auxiliary treatments for mitigating water stress.

Differences between means that do not share the same letter are significant ($\alpha=0.05$).

Auxiliary treatments to mitigate water stress had a highly significant effect on plant height ($F=28.83$, $p<0.001$). On the other hand, the type of substrate did not affect height growth ($F=0.97$, $p=0.378$); however, the interaction of the auxiliary treatment with the substrate was significant ($F=3.14$, $p=0.005$).

For the three types of substrate, the irrigation treatment improved height growth significantly, compared to the control (Figure 4). In plants grown in compost, irrigation yielded superior results to those obtained with hydrogel application. The hydrogel treatments did not produce a significant increase in height in relation to the control, except for the treatment with four grams of hydrogel in the plants cultivated with loam (Figure 4).

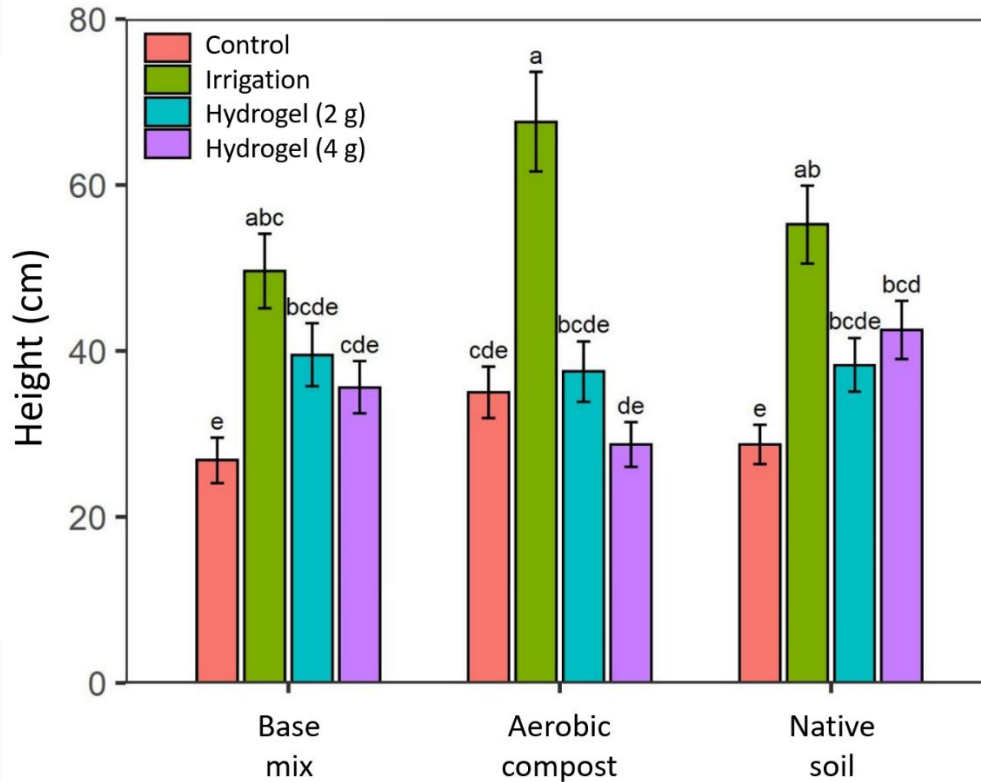


Figure 4. Mean heights \pm SE in *Enterolobium cyclocarpum* (Jacq.) Griseb. plants ten months after establishment, grown in three types of substrates and planted with different auxiliary treatments to mitigate water stress. Differences between means that do not share the same letter are significant ($\alpha=0.05$).

Discussion

The present study demonstrated that the type of substrate with which *E. cyclocarpum* plants are grown in the nursery influences survival at early age of field establishment. On the other hand, the application of auxiliary treatments for water

stress mainly affected growth, although their effect also depended on the type of substrate used in the nursery.

In plant production, the substrate is the element that supports the plant. The type of substrate affects growth and root architecture, and also influences the cohesion and integrity of the root ball (Landis, 1990). This latter aspect is considered an important trait for the quality of nursery-grown plants (Pemán *et al.*, 2017), since proper root ball integrity or conformation has a positive effect on plant survival, particularly on sites with degraded soils (Grossnickle and El-Kassaby, 2016).

In the present study, the native soil proved more favorable to the survival of *E. cyclocarpum* than the other substrates. In a visual inspection, better root ball conformation was observed with the native soil, which possibly improved the ability of the plants to survive. Other characteristics of local substrates, such as additional nutrient content and beneficial microorganisms, can improve plant performance (Jacobs and Landis, 2014). The soil is widely used in the production of plants of other forest species, with good results in variables indicating plant quality (López *et al.*, 2018; Reyes *et al.*, 2018). However, the physical and chemical characteristics of these substrates vary over time and between locations, which limits the standardization and continuous improvement of cultural practices in nurseries (Bakry *et al.*, 2012).

An important finding was the positive relationship between plant diameter and the probability of survival. This effect highlights the importance of considering diameter as a basic indicator of *E. cyclocarpum* survival in the first years of field planting. This result is consistent with studies showing that plants with larger diameters tend to survive better than those with smaller diameters (Orozco *et al.*, 2010; Grossnickle, 2012). In this regard, it has been suggested that the diameter has a direct relationship with certain characteristics of the root system, such as volume or architecture (Jacobs *et al.*, 2005). Furthermore, plants with larger diameters are

likely to have more carbohydrate and nutrient reserves (Tsakalidimi *et al.*, 2013), which, in turn, influences the probability of survival in the field. In addition, plants with larger diameters have greater resistance and resilience to physical damage caused by biotic and abiotic agents.

E. cyclocarpum inhabits the dry tropical forest and is drought tolerant (Foroughbakhch *et al.*, 2006; Laborde and Corrales-Ferrayola, 2012). In this sense, it is likely that the application of some auxiliary treatment to mitigate water stress does not have a significant impact on survival rates, as observed in the present study. However, in the research documented here, it is possible that the plants were not exposed to water stress given the high rainfall at the planting site. On the other hand, in juvenile stages, *E. cyclocarpum* exhibits high growth rates (Foroughbakhch *et al.*, 2006; Rocha *et al.*, 2018), and its performance increases considerably under favorable humidity and fertility conditions (Craven *et al.*, 2007; Pineda-Herrera *et al.*, 2017). Therefore, this may explain why the positive effect of the application of the auxiliary treatment for mitigating water stress manifested in the growth rather than in the survival of the plants.

Irrigation was the auxiliary treatment that increased growth the most, compared to the control. Hydrogel application did not significantly improve plant growth. However, in plants cultivated with loam, more growth in diameter was observed with the application of hydrogel.

The main benefit of hydrogels is to increase the moisture-holding capacity of the soil or growing medium in which they are incorporated (Chen *et al.*, 2004). However, the effectiveness of these polymers will depend on multiple factors such as the type of polymer, particle size, dosage, amount of water available, application method, among others (Crous, 2017). Also, the physical and chemical properties of the soil often affect the effectiveness of hydrogels (Agaba *et al.*, 2010; Crous, 2017). In this sense, the variable response of the plants to the hydrogel application could be

related to the differences in the physicochemical characteristics of the substrates. However, more research is needed to study these relationships in depth.

Conclusions

The authors accept the hypothesis that the type of substrate influences the survival and growth of the *Enterolobium cyclocarpum* plants, and that native soil promotes better performance. Diameter maybe an indicator of survival of *E. cyclocarpum* at early ages after planting. Auxiliary treatment to mitigate water stress impacts growth rather than survival. However, it is advisable to consider the rainfall and humidity conditions of the planting site in order to determine the need for the application of this type of treatment. The results contribute to promoting a better performance of the study species in commercial plantations or reforestations.

Acknowledgements

The National Council of Science and Technology (*Conacyt*) for the scholarship granted to the first author to carry out his postgraduate studies.

Conflict of interest

The authors declare that they have no conflict of interest.

Contribution by author

Adrián Botello Montoya: fieldwork, statistical analysis and drafting of the manuscript; Eduardo Alanís Rodríguez: research design, project supervision and drafting of the methodology section; José Ángel Sigala Rodríguez: statistical analysis, generation of graphs and drafting of the results section; Jesús Eduardo Silva García: fieldwork and drafting of the discussion section; Luis Daniel Ruiz Carranza: management of financial resources, fieldwork and drafting of the introduction. All authors revised and approved the final document.

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