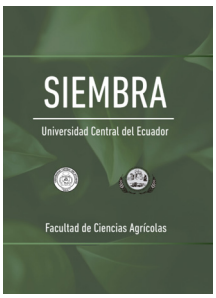
Evaluation of alternative rooting agents on root growth of wasp flower (Hibiscus rosa-sinensis L.) cuttings under nursery conditions



Evaluación de enrraizadores alternativos sobre el crecimiento radicular de esquejes de flor de avispa (Hibiscus rosa-sinensis L.) bajo condiciones de vivero

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Abstract

The use of alternative tree and shrub species such as Hibiscus rosa sinensis L, for feeding ruminants, can provide high amounts of feed biomass and high protein content. The most commonly used propagation methods for the production of seedlings of this species are by grafting, layering and cuttings, with the latter method standing out, because it allows obtaining a greater number of seedlings per branch in less time. The objective of the research was to evaluate the effect of alternative rooting agents on wasp flower (*H. rosa-sinensis L.*) cuttings, under nursery conditions. Five rooting agents were evaluated consisting of *Aloe vera*, cinnamon, *Aloe* + molasses + egg (A+M+E), *Moringa oleífera* and the antagonistic fungus *Trichoderma sp*, plus a control consisting of the addition of water. The variables evaluated were number of roots per plant, root length, number of regrowths, fresh root weight per plant, dry root weight per plant, root biomass and survival percentage. The data were organized in a Microsoft Excel 2013 spreadsheet, then analyzed in the statistical program InfoStat 2020. The treatment with *Trichoderma sp* favors the vegetative growth of *H. rosa-sinensis*, followed by the A+M+E, *Moringa* and cinnamon treatments. The treatments with the highest percentage of survival were *Moringa*, cinnamon, *Aloe*, and *Trichoderma sp*, considered as excellent, while the A+M+H and control treatments were considered good.

Keywords: biomass, survival, *Trichoderma sp*, *Aloe vera*, vegetative growth

Resumen

El uso de las especies alternativas de árboles y arbustos como son *Hibiscus rosa sinensis* L, para la alimentación de rumiantes, puede proporcionar altas cantidades de biomasa alimenticia y alto contenido de proteína. Los métodos de propagación más utilizados para la producción de plántulas de esta especie son por injerto, acodo y esquejes, destacándose este último método, porque permite obtener un mayor número de plántulas por rama en menor tiempo. El objetivo de la investigación fue evaluar el efecto de enrraizadores alternativos sobre esquejes de flor de avispa (*H. rosa-sinensis* L.), bajo condiciones de vivero. Se evaluaron cinco enrraizadores que consistieron en *Aloe vera*, canela, *Aloe* + melaza + huevo (A+M+H), *Moringa oleifera* y el hongo antagonista *Trichoderma*

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sp, además de un testigo que consistió en la adición de agua. Las variables evaluadas fueron número de raíces por planta, longitud de raíces, número de rebrotes, peso fresco de raíz por planta, peso seco de raíz por planta, biomasa de raíces y porcentaje de sobrevivencia. Los datos fueron organizados en una hoja de cálculo de Microsoft Excel 2013, posteriormente fueron analizados en el programa estadístico InfoStat 2020. El tratamiento con *Trichoderma* sp favorece el crecimiento vegetativo de *H. rosa-sinensis*, seguido de los tratamientos A+M+H, *Moringa* y canela. Los tratamientos con mayor porcentaje de sobrevivencia fueron *Moringa*, canela, *Aloe* y *Trichoderma* sp, considerados como excelentes, mientras que los tratamientos A+M+H y testigo fueron considerados buenos.

Palabras clave: biomasa, sobrevivencia, *Trichoderma* sp, *Aloe vera*, crecimiento vegetativo

1. Introduction

Pastures and forages are the primary food source for ruminants in tropical areas. In these regions, forage yield and nutritional value vary throughout the year due to climate and poor land management. In this scenario, the year seasons are an essential issue. During the rainy season, there is a surplus of forage, while during the dry season, the availability of forage is low. In this sense, the use of alternative species of trees and shrubs *(Hibiscus rosa sinensis)* can provide high amounts of food biomass (30 t ha^{-1} y 21 t ha^{-1} $year^{-1}$) and high protein content (19 %), compared to tropical grasses (8%). This makes them an option as food for ruminants during the dry season (Cruz Hernández et al., 2019).

Non-legume species, such as *Morus alba*, *Hibiscus rosa-sinensis,* and *Trichanthea gigantea*, show high nutritional potential. Their high protein concentration and rapid degradation in the rumen suggest that their inclusion in low-quality diets could improve efficiency by using and maintaining adequate production levels (Flores et al., 1998).

The propagation methods most used to produce seedlings of this species *(Hibiscus rosa sinensis)*, known by various common names such as Chinese rose, Chinese Hibiscus, wasp flower, or simply *Hibiscus*, is achieved by grafting, layering, and cuttings. This last method is highlighted because it allows for obtaining more seedlings per branch in less time (Lorenzi & Souza, 2008).

Root formation is vital to absorbing and conducting water plus dissolved minerals, accumulating nutrients, and anchoring the plant to the soil. Hormones produced naturally or synthetically are alternatives to have greater success in the attachment of vegetative parts to help the proliferation and formation of a sound root system that allows the growth and development of a new plant. The applications of synthetic rooting hormones are successful but expensive, so it is necessary to improve knowledge of vegetative propagation using natural substances (León Araujo, 2011; Jamal Uddin et al., 2020).

Direct-sowing *H. rosa sinensis* plants have poor root systems during the first 60 days, so the absorption of water or nutrients at this time is very limited. Furthermore, its orientation is superficial plagiogravitropic. This response may be due to the fragility of the root system, soil texture, and very high humidity gradients at the surface level of the soil (Cuéllar & Arrieta Herrera, 2010).

Organic rooting products can increase the root system and, therefore, the nutrient absorption capacity to achieve efficient technological management of pastures and forages. In this way, the plants will have greater anchorage, and thus, lodging can be avoided (Vicuña Molina, 2015). Considering the previous information, the present research work evaluates alternative rooting agents to commercial ones, which contribute to generating good growth of *Hibiscus* cuttings under nursery conditions.

2. Materials and Methods

2.1. Experiment Location

The experiment was carried out on the Santa Rosa farm of the Universidad Nacional Agraria. The experimental area is located at the geographical coordinates, “12°08’15.9” north longitude and 86°10’05.9” west longitude, with an altitude of 56 m a.s.l. (Instituto Nicaragüense de Estudios Territoriales [INETER], 2022), The period evaluated was from September 2022 to January 2023.

2.2 Experiment design

The experiment was carried out using a completely randomized design (CRD) with six treatments: T1-*Aloe vera*; T2-*Aloe vera* + molasses + egg; *Cinnamomum verum* T3; T4-*Trichoderma sp*; *T5-Moringa oleifera*; T6-witness. One hundred cuttings were used per treatment, and each one constitutes an experimental unit, for a total of 600 cuttings placed in an area of approximately 32.2 m^2, one meter wide by two meters long and one meter wide from separation.

2.3. Description of treatments

Six treatments were evaluated consisting of *Aloe vera*, *Cinnamomum verum*, aloe + molasses + egg, *Moringa oleifera, Trichoderma* sp and control (water):

- T1: *Aloe vera*: Large and vigorous leaves of the *Aloe* plant were selected, washed, and, with the help of a small knife, the epidermis was removed to obtain the gel. Impurities were removed, and finally, the gel was deposited in a container for later. The cuttings were submerged in the *Aloe* for five minutes to impregnate the lower part of the cutting on the stem and placed in nursery bags containing substrate (El-Ahmir et al., 2021).
- T2: *Aloe vera* + molasses + egg: The same procedure described previously in T1 was carried out to obtain the *Aloe* gel. Then molasses and eggs were added (only the yolks were used). The ingredients were mixed and evenly integrated. The cuttings were soaked for three minutes and placed in nursery bags containing substrate (Araúz Meza & Luquéz Díaz, 2020).
- T3: *Cinnamomum verum*: 30 g of cinnamon per liter of water were used, and it was left to rest for one hour; after that time, it was filtered, and the cuttings were immersed for three minutes before sowing them in nursery bags (El-Ahmir et al., 2021).
- T4: *Trichoderma sp*: 12.5 g of *Trichoderma* were used in 10 L of water (a commercial dose of 250 g of the fungus in rice substrate per hectare). The cuttings were then immersed for five minutes and transferred to nursery bags containing substrate. This microorganism has been described as a root growth promoter in annual and perennial crops.
- T5: *Moringa oleifera*: Healthy leaves of the plant were selected in an equivalent of 500 g, liquefied with three liters of water, and allowed to rest for five hours. Subsequently, the cuttings were immersed in the solution for 20 min and placed in substrate nursery bags (Pérez-Gómez et al., 2019).
- T6: Without rooting solution (absolute control): The cuttings were immersed in water for three minutes before being planted in nursery bags containing substrate.

Each treatment was applied to 100 cuttings of *H. rosa sinensis*; 8 x 12-inch bags were used, containing substrate composed of soil from the surrounding area and rabbit excreta. Randomization was carried out to distribute the blocks. Subsequently, the lower parts of the cuttings were immersed in the rooting agents for variable times, and they proceeded to sow (Table 1). The data were recorded 30 days (11/03/20), 60 days (12/02/2022), and 90 days (01/03/2023) after planting the cuttings.

2.4. Tested variables

The variables were taken at 30 days, 60 days, and 90 days after planting the cuttings; on each date, a random sample of 10 cuttings per treatment was taken, giving a total of 60 cuttings to the 30, 60, and 90 days. The variables tested were:

- Root length (*RL*): A ruler measured each root's length (cm) 30, 60, and 90 days after planting the cuttings.
- Root thickness (*RT*): A vernier measured each root's thickness (mm) emitted 30, 60, and 90 days after planting the cuttings.
- Number of roots per cutting (*NRC*): The number of roots per cutting was counted 30, 60, and 90 days after sowing.
- Number of secondary roots (*NSR*): The number of secondary roots was counted for each root for each cutting at 30, 60, and 90 days after sowing.

Table 1. *Treatments evaluated on Hibiscus cuttings in the nursery stage.*

Treatment	Rooting solution	Composition	Time (min)
T1	*Aloe vera*	2.000 ml of *Aloe*	5
T2	A+M+E	*Aloe* 200 ml + molasses 500 ml + egg 200ml	3
T3	*Cinnamomum verum*	30 g per L of water	3
T4	*Trichoderma* sp	12,5 g + 10 L of water	5
T5	*Moringa oleifera*	*Moringa* 500 g + water 3.000 ml	20
T6	Control	Water	5

- Root dry weight per cutting (*DW*): The roots were placed in an oven at 65oC for 48 hours to obtain the dry weight for each plant individually.
- Percentage of root biomass (*%RB*): To determine the root biomass, the percentage of humidity was taken into account. For this, the fresh weight and dry weight of the roots were used. Once the percentage of humidity was calculated, the biomass was determined by multiplying the roots' fresh weight by the humidity percentage. Using the mathematical formulas (equations [1] and [2]) described by Ávila Guevara (2016):

$$\%B = Pf - \left(\frac{H}{100}\right) \quad [1]$$

where:
  - *H***:** humidity percentage (%)
  - *FW***:** fresh weight (kg)
  - *DW***:** dry weight (kg)

$$\%B = Pf - \left(\frac{H}{100}\right) \quad [2]$$

where:
  - *%B***:** biomass (kg)
  - *FW***:** fresh weight (kg)

- Survival percentage *(%S*): It was evaluated after the first week of establishment in nursery bags. The variable was evaluated for each treatment using the live and dead cuttings count. During the trial, equation [3] and survival category (Table 2), proposed by Saavedra Miranda and Gutiérrez Gonzáles (2014), were taken as a reference for the calculation.

$$\%S = \frac{n2}{n1} * 100 \quad [3]$$

where**:**
  - %S**:** Survival rate
  - n1**:** Initial cuttings
  - n2: Live cuttings found at the end of the trial

Table 2. *Survival category expressed in percentages.*

Category	Survival rate (%)
Excellent	90 -100
Good	75 - 90
Regular	60 - 75

2.5. Data Analysis

The data were organized in a Microsoft Excel 2013® spreadsheet. Subsequently, the variables root length, root thickness, number of roots per cutting, number of secondary roots, fresh weight of root per cutting, dry weight

of root per cutting, and percentage of biomass were analyzed through a variance (ANOVA) as a completely randomized design (CRD) with a factorial structure of 6 x 100 (6 treatments x 100 experimental units), for which the model in equation [4] was used.

$$Yij = \mu + Ti + \varepsilon ij = \ 100; j = 6; T = \ 600 \quad [4]$$

where:
- *Yij*: represents the i-th experimental unit and j-th treatment
- μ: represents the general average
- *Ti*: is the effect of the i-th treatment
- *Eij*: represents the experimental error.

Mean separations were performed using Tukey (Quinn & Keough, 2002). Descriptive analysis was performed on the survival percentage variable.

3. Results and Discussion

3.1. Effect of rooting agents on vegetative growth variables in H. rosa sinensis at 30 days

The analysis of variance for the vegetative growth variables for several secondary roots (NSR), root length (RL), and fresh weight (FW) 30 days after planting cuttings showed significant statistical differences between treatments (p = 0 .0014, p = 0.0052 and p = 0.0435), respectively, and Tukey's mean separation test ($\alpha = 0.05$) indicated that the treatment with *Aloe vera* plus molasses plus egg (A+M+E) presented the means with the highest values, in NSR (12.2). However, there was no significant difference between cinnamon, *Moringa*, and absolute control.

The *Aloe vera* and *Trichoderma* sp treatments showed the means with the lowest values for the NSR variable. Regarding the RL, the A+M+E treatment showed mean values of 5.58 cm, but this was not very different from the cinnamon, absolute control, *Moringa*, and *Trichoderma* sp treatments. The *Aloe vera* treatment presented lower RL with 1.44 cm, which is not different from the absolute control, *Moringa*, and *Trichoderma sp*. treatments (Table 3).

Table 3. *Effect of rooting solutions on H. rosa sinensis 30 days after planting cuttings.*

Treatment	Physiological variables*		
	RL (cm)	NSR	FW (mg)
Aloe	1,44 b	1,06 c	5,14 ab
Trichoderma sp	2,22 ab	2,16 bc	2,14 b
A+ M+ E	5,58 a	12,20 a	11,24 a
Moringa	4,28 ab	8,56 abc	5,82 ab
Cinnamon	5,18 a	10,50 ab	5,46 ab
Control	4,44 ab	5,42 abc	3,56 ab
R^2	0,48	0,54	0,36
CV	45,55	64,14	75,64
p-value	0,0052	0,0014	0,0435

* RL: Length of roots, NSR: Number of secondary roots, FW: Fresh weight. Means with a common letter between columns are not significantly different (p > 0.05).

The A+M+E treatment obtained the highest FW values, 11.24 mg, which were not significantly different from the *Moringa*, cinnamon, *Aloe vera*, and control treatments. The *Trichoderma* sp treatment showed the lowest FW values, 2.14 mg, which were not significantly different from the *Moringa*, cinnamon, *Aloe vera*, and absolute control treatments.

3.2. Effect of rooting agents on vegetative growth variables in H. rosa sinensis at 60 days

The RT, RL, NSR, FW, DW variables and %RB 60 days after planting the cuttings showed significant statistical differences between treatments ($p = 0.0007$, $p = 0.0005$, $p = 0.0001$, $p = 0.0044$, $p = 0.0002$ and $p = 0.0002$, respectively). Tukey's mean separation test ($\alpha = 0.05$) indicated that the *Trichoderma sp* treatment presented the means with the highest value in RT (1.58). However, it did not show a significant difference concerning A+M+E, *Moringa*, cinnamon, and absolute control. The *Aloe* treatment showed the means with the lowest value for the RT variable. The *Trichoderma s*p treatment presented the mean with the highest value in the case of RL (10.52). However, it did not show a significant difference with the control. Treatment with *Aloe* showed the means with the lowest value for the RL variable.

The control treatment presented the means with the highest NSR value (34.0 cm). However, there were no significant differences concerning *Trichoderma sp*. The *Aloe* treatment showed the means with the lowest value for the NSR variable. However, there are no significant differences between A+M+E, Moringa, and cinnamon. The *Trichoderma* sp treatment presented the means with the highest FW value (137.02 mg). However, there were no significant differences concerning A+M+E, Moringa, cinnamon, and the control. The *Aloe* treatment showed the means with the lowest value for the FW variable. However, it is not very different from A+M+E, *Moringa*, and cinnamon (Table 4).

Table 4. *Effect of rooting solutions on H. rosa sinensis 60 days after planting cuttings.*

Treatment	Physiological variables *					
	RT (mm)	RL (cm)	NSR	FW (mg)	DW (mg)	% RB
Aloe	0,70 b	3,94 c	7,66 c	4,12 b	2,26 c	3,76 c
Trichoderma sp	1,58 a	10,52 a	22,90 ab	137,02 a	34,50 ab	188,36 a
A+ M+ E	1,18 ab	7,98 abc	20,74 bc	36,68 ab	14,06 bc	36,20 bc
Moringa	1,28 a	6,62 abc	14,62 bc	35,74 ab	12,56 bc	35,20 bc
Cinnamon	1,32 a	6,22 bc	17,74 bc	43,90 ab	14,96 bc	43,42 bc
Control	1,08 ab	9,96 ab	34,00 a	148,58 a	55,12 a	148,06 ab
R^2	0,57	0,58	0,64	0,49	0,62	0,61
CV	21,97	28,54	34,45	92,24	68,40	79,32
p-value	0,0007	0,0005	0,0001	0,0044	0,0002	0,0002

* GR: Root thickness, RL: Root length, NSR: Number of secondary roots, FW: Fresh weight, DW: Dry weight, %RB: Percentage of biomass. Means with a common letter between columns are not significantly different ($p > 0.05$).

The control treatment presented the means with the highest value in DW (55.12 mg). However, there were no significant differences concerning *Trichoderma sp*. The *Aloe* treatment did not show significant differences for DW (2.26 mg). However, there are no significant differences between A+M+E, *Moringa*, and cinnamon. The *Trichoderma* treatment presented the means with the highest value in %RB (188.86%). However, there were no significant differences between the absolute control and the absolute control. The *Aloe* treatment showed the means with the lowest value for the %RB variable (3.76%). However, there are no significant differences between A+M+E, *Moringa*, and cinnamon (Table 4).

3.3. Effect of rooting agents on vegetative growth variables in H. rosa sinensis at 90 days

The FW, DW variables, %H, and %RB 90 days after planting the cuttings showed significant statistical differences between treatments ($p = 0.0001$, $p = 0.0001$, $p = 0.0243$, and $p = 0.\ 0001$), respectively. Moreover, the Tukey separation test ($\alpha = 0.05$) indicated that the *Moringa* treatment presented the means with the highest values in FW (284.63 mg). However, there were no significant differences in relation to *Trichoderma sp*, A+ M+ E, and control.

The *Aloe* treatment showed the means with the lowest PF value. The Moringa treatment presented the means with the highest DW value (118.88 mg). However, the control, *Moringa*, and *Trichoderma* sp did not show significant differences. The aloe treatment showed the lowest value (44.53 mg) for the DW variable; however, it is not different from the cinnamon treatment (Table 5).

Table 5. *Effect of rooting solutions on H. rosa sinensis 90 days after planting cuttings.*

Treatment	Physiological variables *			
	FW (mg)	DW (mg)	% H	% RB
Aloe	44,53 c	27,35 d	33,73 b	44,18 c
Trichoderma sp	206,75 a	98,77 ab	37,07 ab	206,40 a
A+ M+ E	187,77 ab	80,98 bc	42,88 a	187,32 ab
Moringa	284,63 a	118,88 ab	41,55 ab	284,17 a
Cinnamon	65,22 bc	43,93 cd	32,82 b	64,88 bc
Control	293,80 a	136,63 a	38,13 ab	293,42 a
R^2	0,68	0,75	0,34	0,68
CV	39,98	29,34	15,06	40,04
p-value	0,0001	0,0001	0,0243	0,0001

* FW: Fresh weight, DW: Dry weight, %H: Percentage of humidity, %RB: Percentage of root biomass. Means with a common letter between columns are not significantly different ($p > 0.05$).

The A+M+E treatment presented the means with the highest values in %H (42.88%). However, there were no significant differences between *Trichoderma* sp, *Moringa*, *Aloe*, and control. The cinnamon treatment showed the means with the lowest value in %H; it was not statistically different from the *Trichoderma* sp, *Moringa*, *Aloe*, and control treatments. The *Moringa* treatment presented the means with the highest %RB (284.17%). However, *Trichoderma s*p, A+ M+ E, and control did not show significant differences. The *Aloe* treatment showed the means with the lowest value in %RB (Table 5).

This study's results show that treatment with *Trichoderma* sp can favor the vegetative development of *H. rosa-sinensis*, followed by the *Moringa*, A+M+E, and cinnamon treatments. The factors that could influence the results of this research are the allometric growth of the plants, lighting effect, water, and substrate. However, it is necessary to carry out these types of studies to deepen the knowledge about the variables evaluated. There is little information, and its use has been limited to forage and ornamental food in Nicaragua.

Paz (2019), in his morphological field study in *Hibiscus*, identified physiological resistances that the species present to counteract the water stress generated during drought. This action arises as a mechanism of foliar readjustment to guarantee a water balance and thus not affect the rest of the foliage.

Cuéllar and Arrieta Herrera (2010) demonstrated that during the growth phases of *H. rosa-sinensis* plants, a 90-day nursery phase is necessary, a time in which the plant shows better morphological conditioning in height and vigor. The results for root and shoot weights showed that under nursery conditions, plants maintain an allometric balance in weight accumulation for up to 84 days (0.86±0.31 for the root and 2.71±0.22 for the shoot), presenting statistical differences after 91 days, where the accumulation of dry matter in the shoot increases substantially up to 13.87 ± 0.21. it also concludes that the species *H. rosa-sinensis* necessarily requires a nursery phase of up to 98 or 105 days.

The biomass productivity of a plant community can be estimated as a linear function of the amount of radiation intercepted by *H. rosa-sinensis*. In species such as *H. rosa-sinensis*, productivity depends more on variations in the amount of incident solar radiation accumulated in a certain period.

Chowdhuri and Sadhukhan (2019), using synthetic rooting agents, mention that naphthaleneacetic acid in concentrations of 1,000 to 3,000 ppm can be used for rooting *H. rosa sinensis*, but higher doses are beneficial during the rainy season in the subtropical zone. In this study, the second-best growth regulator was indole-3-butyric acid at 3,000 ppm as a rooting agent.

3.4. Survival percentage of H. rosa sinensis under nursery conditions

At 120 days after sowing the complaints, it was found that the treatments with the highest %S were *Moringa* (97%), cinnamon (96%), *Aloe* (91%), and *Trichoderma* sp (90%), which were considered excellent. The treatment with the lowest %S was the A+M+E treatment (89%) and control (88%), which were considered good (Figure 1).

The substrates must provide the necessary elements for growth: water, air, and nutrients. Currently, mineral fertilizers can precisely provide the latter to the crop. Water and air availability depends on the substrate's physical and mechanical properties.

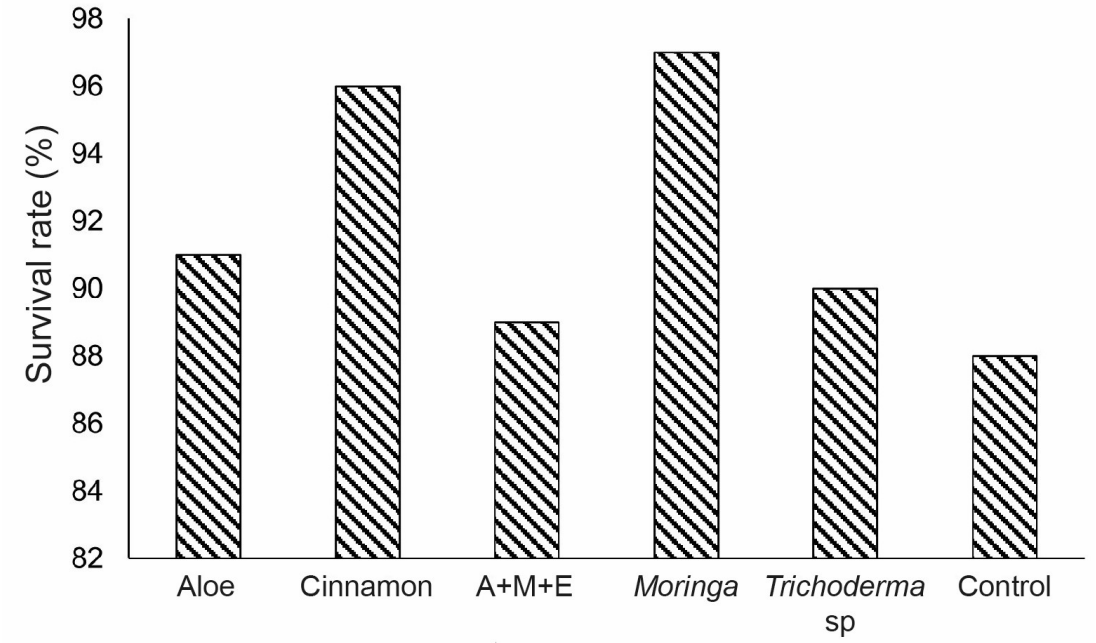

Figure 1. Survival percentage of *H. rosa sinensis* in the nursery 120 days after sowing.

4. Conclusions

Treatment with *Trichoderma* sp favors the vegetative growth of *H. rosa sinensis* 60 days after sowing, followed by the *Moringa*, A+M+E, and *Cinnamomum verum* treatments. The treatments with the highest survival percentage were *Moringa*, *Cinnamomum verum*, *Aloe*, and *Trichoderma sp*, which were considered excellent, while the A+M+E and control treatments were considered good.

Author contributions

- Cristhian Georgina Benavides Gómez: formal analysis, investigation, methodology, visualization.
- Julissa Edith Rugama Picado: formal analysis, investigation, methodology, visualization
- Isaías Ezequiel Sánchez Gómez: conceptualization, formal analysis, project administration, investigation, visualization, writing – review and editing.
- Eliezer Hazael Lanuza Rodríguez: conceptualization, formal analysis, research, visualization, writing – review & editing.
- Marcos Antonio Jiménez Campos: investigation, supervision, visualization, writing – review & editing.

Ethical implications

Ethics approval not applicable

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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