

Influence of *Rhizophagus intraradices* (Schenck & Sm.), Chemical and Organic Fertilization on the Growth of *Swietenia humilis* Zuccarini in Nursery Conditions

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ABSTRACT

Objective: To evaluate the growth of *Swietenia humilis* Zuccarini (caobilla) seedlings when grown in different substrates and biofertilized with and without *Rhizophagus intraradices* in combination with chemical fertilization.

Design/Methodology/Approach: Caobilla seeds were collected in Huehuetán, Chiapas, and once germinated, were transplanted into plastic bags filled with a soil-based substrate supplemented with 30% cattle manure. The following treatments were established: (1) control; (2) cattle manure; (3) cattle manure + *R. intraradices*; (4) 100N-50P; (5) 100N-50P + *R. intraradices*; (6) 50N-25P + *R. intraradices*. Each treatment had five replicates and was arranged in a completely randomized design. Morphological variables were recorded every 28 days, and a final destructive sampling was performed at 121 days after transplanting (DAT) to determine dry biomass. Data were analyzed using analysis of variance (ANOVA).

Results: The morphological and physiological variables indicated an increase in the growth of caobilla when cattle manure was added to the substrate and plants were biofertilized with *R. intraradices*.

Study Limitations/Implications: The plant response may vary if caobilla is biofertilized with a different species of arbuscular mycorrhizal fungus.

Findings/Conclusions: The greatest growth in the biomass of morphological and physiological components of *S. humilis* Zucc. was observed in the treatment with cattle manure combined with *R. intraradices*. The highest chemical fertilization dose also promoted biomass accumulation, particularly at the end of the evaluation period.

Keywords: caobilla, arbuscular mycorrhizal fungi, alternative substrates, biomass.

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INTRODUCTION

Swietenia humilis Zuccarini, commonly known as caobilla, is a species with high commercial demand due to the quality of its wood. However, its natural populations have significantly declined. Therefore, for the successful establishment of this species in areas with low population density, the production of high-quality nursery-grown plants is essential prior to field transplantation. A crucial first step in this process involves the



use of high-quality seeds and appropriate substrates for germination. Without these considerations, reforestation efforts are likely to be unsuccessful. Traditionally, the substrate used for this purpose is imported peat moss (*Sphagnum magellanicum*), which is costly. This situation highlights the need to explore alternative local options, including organic and chemical amendments, capable of promoting rapid and vigorous plant growth in nursery conditions. One such alternative involves the incorporation of cattle manure, which is abundantly available in the coastal region of Chiapas, Mexico. Additionally, the growth of forest seedlings has been improved through the inclusion of arbuscular mycorrhizal fungi (AMF) during planting. These fungi establish symbiotic relationships with host plants across nearly all ecosystems (Strack *et al.*, 2003), enhancing plant growth through nutrient and water transport (Redecker, 2000; Aguirre-Medina *et al.*, 2016; Aroca *et al.*, 2011). Although AMF are not host-specific, there is evidence of preferential associations between certain fungal species and specific plant hosts or fungal isolates (Anzueto-Herón *et al.*, 2023; Aguirre-Medina *et al.*, 2023). The beneficial effects of AMF symbiosis in promoting plant growth have been reported in various perennial crops, where it helps reduce the time needed for plants to reach transplant readiness. This has been demonstrated, for example, in robusta coffee (*Coffea canephora* Pierre ex Froehner) (Ibarra-Puón *et al.*, 2014). Furthermore, mycorrhizal symbiosis has been shown to increase the accumulation of key nutrients such as phosphorus and nitrogen in plant tissues (Aguirre-Medina *et al.*, 2012). Based on this background, it is expected that the application of arbuscular mycorrhizal fungi to caobilla seeds in the nursery stage will promote early-stage growth and reduce the time required for seedlings to achieve the stem thickness necessary for successful field establishment. Therefore, the aim of this research was to evaluate the growth of *Swietenia humilis* when amended with cattle manure and biofertilized with and without *Rhizophagus intraradices*, in combination with chemical fertilization.

MATERIALS AND METHODS

Study site

The research was conducted at the nursery of the Faculty of Agricultural Sciences, Universidad Autónoma de Chiapas (UNACH), located in Huehuetán, Chiapas, Mexico, at an altitude of 44 m. The site is geographically positioned between 15° 00' and 15° 30' N latitude and 94° 30' and 95° 00' W longitude. The climate is classified as Am (w') i g, corresponding to a warm humid climate with summer rains, an average annual precipitation of 2500 mm, and maximum and minimum temperatures of 38 °C and 15 °C, respectively, with an average temperature of 25.4 °C (García, 2004). The substrate consisted of fluvisol soil (INEGI, 2004) collected from Ejido Huehuetán, Chiapas, mixed with 30% cattle manure ground using a hammer mill (Swrssrnex model 610120) and solarized for 72 hours. The substrate's physical-chemical properties were as follows: sandy loam texture with 80.76% sand, 13.36% silt, and 5.88% clay; 2.63% organic matter; pH 5.78; 0.13% nitrogen; 14.1 ppm phosphorus; 64.2 ppm potassium; 474.00 ppm calcium; 58.00 ppm magnesium; 102.50 ppm sodium; and electrical conductivity (EC) of 0.05.

Rhizophagus intraradices (Schenck & Sm.) was obtained from the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) at the Rosario Izapa

Experimental Field. The inoculum had a concentration of 40 spores per gram of soil and 95% root colonization in the host plant *Brachiaria brizantha* (Hochst. ex A. Rich.) according to the product data (Micorria INIFAP®). The experimental unit was a container with a single plant. Seedlings were irrigated with deep well water four times per week using a manual watering can, with subsequent irrigation depending on environmental conditions.

Sowing and application of microorganisms and chemical fertilizer

Caobilla seeds were collected from a selected tree on the premises of the Faculty of Agricultural Sciences, Huehuetán Campus IV, chosen for its dense canopy and absence of diseases and pests. For germination, seeds were coated with carboxymethylcellulose and inoculated with *R. intraradices* biofertilizer at 4% of the seed weight. Biofertilized seeds were sown in trays with 24 cones of 150 cm³ each, measuring 19.5 cm in height and 4.0 cm in upper diameter, at a depth of 3.0 cm. After 10 days, seedlings were transplanted into black plastic bags (25×35 cm) with a 1 kg soil capacity and placed on metal benches. The bags were perforated at the bottom to ensure proper drainage.

Treatments, replications, and experimental design

The following treatments were evaluated: (1) control, (2) cattle manure, (3) 100N+50P, (4) cattle manure + *R. intraradices*, (5) 100N+50P + *R. intraradices*, and (6) 50N+25P + *R. intraradices*. All treatments were arranged in a completely randomized block design with five replicates. Destructive sampling was performed at 121 days after transplanting (DAT).

Evaluated variables

Morphological variables (plant height, stem diameter, and number of leaves) were recorded at 14, 28, 42, 56, 70, 84, 98, and 112 DAT. Physiological variables (dry weight of aerial and root components) were measured at 112 DAT. Root and shoot biomass were weighed using a semi-analytical balance (Ohaus Adventurer Pro, USA) after drying in a forced-air oven at 75-80 °C until reaching constant weight. The root-to-shoot ratio was determined following Boonstra (1931).

Statistical analysis

The results were analyzed using SAS software version 8.1 (SAS Institute, 1999-2000) with a completely randomized design. Treatment means were compared using Tukey's test ($P \leq 0.05$), and graphs were generated using Sigma Plot (version 11.0).

RESULTS AND DISCUSSION

Plant height exhibited consistent changes starting at 56 days after transplanting (DAT), with the treatments that induced the greatest height being cattle manure alone, cattle manure combined with seed biofertilization using *R. intraradices*, and the chemical fertilization treatment with 100N+50P (Table 1).

Plants grown in the soil substrate supplemented with cattle manure and biofertilized with *R. intraradices* showed a 5% increase in height at the final sampling and were

Table 1. Mean comparisons of morphological components in *Swietenia humilis* (caobilla) biofertilized with *Rhizophagus intraradices* in interaction with cattle manure and chemical fertilization under nursery conditions.

	Time (days)	Witness	Bovine manure	Bovine manure + <i>R. intraradices</i>	100 _N -50 _P	100 _N -50 _P + <i>R. intraradices</i>	50 _N -25 _P + <i>R. intraradices</i>	**CV (%)
Height (cm.floor ⁻¹)	14	12.7 b*	13.7 ab	15.1 a	12.7 b	11.8 b	12.0 b	8.12
	28	15.0	14.8	16.9	15.7	14.9	15.6	8.5(NS)
	42	17.3	18.1	18.0	17.9	16.4	16.6	5.3(NS)
	56	19.3 b	20.9 ab	22.3 a	21.0ab	19.2 b	20.1 b	5.2
	70	19.7 c	21.5 bc	23.5 a	22.2ab	20.7 bc	21.0 c	4.5
	84	20.8 c	25.1 ab	27.2 a	24.4 b	22.5 bc	21.0 c	5.7
	98	24.7 c	28.3 ab	30.3 a	28.6 a	24.3 c	24.8 bc	6.8
	112	28.3 b	31.4 ab	32.9 a	31.4ab	29.3 ab	28.6 b	6.4
Stem diameter (mm.plant ⁻¹)	14	2.4 ab	2.5 a	2.6 a	2.5 a	2.4 ab	2.1 b	5.8
	28	3.1 ab	3.2 a	3.2 a	2.7 b	3.0 ab	2.9 ab	7.3
	42	3.5	3.6	3.5	3.1	3.4	3.3	6.9(NS)
	56	4.5 b	5.5 a	5.6 a	5.3 a	5.4 a	5.4 a	7.4
	70	7.3 bc	8.0abc	8.6 a	8.2 ab	7.1 cd	6.2 d	6.9
	84	8.3 ab	8.9 a	9.3 a	8.9 a	7.8 bc	7.2 c	6.2
	98	9.4bcd	10.8abc	11.3 a	10.9ab	9.4 cd	8.7 d	7.4
	112	9.9 b	11.5 ab	12.4 a	11.5ab	10.0 b	9.9 b	8.3
Number of leaves.plant ⁻¹	14	2.8 ab	3.6 a	3.2 a	3.8 a	2.0 b	3.2 a	16.6
	28	4.0 b	7.4 a	3.0 bc	3.0bc	2.4 c	2.4 c	17
	42	5.2	5.6	6.0	6.2	5.2	5.2	11.8(NS)
	56	5.6 b	7.2 a	4.8 bc	5.4 b	4.8 bc	3.8 c	13.2
	70	7.6 ab	8.4 a	8.4 a	8.6 a	6.8 b	6.8 b	7.6
	84	7.6 c	8.8abc	9.4 a	9.2ab	7.8 bc	7.4 c	9.1
	98	8.8 ab	8.6 ab	9.4 a	9.6 a	8.0 b	8.8 ab	7.7
	112	9.8 ab	9.6 ab	10.6 a	10.0ab	9.0 b	9.8 ab	7.2

* Values with the same letter within each factor and row are statistically similar according to Tukey's test ($P \leq 0.05$). ** CV=Coefficient of variation.

statistically different ($P \leq 0.05$). The initial lack of response to biofertilization during the first 42 days coincides with the root colonization period of the fungus, during which the benefits of nutrient and water transport to the host plant are still limited. In the other treatments, the early root system was small, with reduced capacity for nutrient uptake and transport. The increase in plant height due to mycorrhizal symbiosis with *R. intraradices* has been documented in several perennial crops, including cacao (Aguirre-Medina *et al.*, 2007), arabica coffee (Anzueto-Herón *et al.*, 2023), and robusta coffee (Ibarra-Puón *et al.*, 2014). Once established, the mycorrhizal fungus uses its hyphal network to explore a larger soil volume, supplying essential nutrients to the host plant, particularly phosphorus (Garza-Cano *et al.*, 2005), nitrogen (Anzueto-Herón *et al.*, 2023), and water, as observed in *Casuarina equisetifolia* (Valdéz *et al.*, 2004).

For stem diameter, biofertilization demonstrated a consistent effect, showing differences from the first evaluation. From 56 DAT until the final measurement, treatments with cattle manure alone, cattle manure combined with *R. intraradices*, and the 100N+50P fertilization formed the leading statistical group, significantly outperforming the other treatments ($P \leq 0.05$). Among these, stem diameter was 8% greater when cattle manure was included in the substrate and seeds were biofertilized with *R. intraradices*. Similar findings were reported in cedar, where increased aerial biomass was observed with biofertilization using inoculum from two tropical ecosystems (Méndez-Cortés *et al.*, 2013). Other forest species, such as teak (*Tectona grandis*) and ronrón (*Astronium graveolens*), also showed increases in stem diameter by 11.8% with mycorrhizal application compared to controls (Hernández and Salas, 2009).

The number of leaves showed differences from the first sampling, with consistent results in treatments using cattle manure alone and cattle manure plus *R. intraradices*. This variable also increased under high chemical fertilization. At 112 DAT, the treatment combining *R. intraradices* and cattle manure resulted in a 9% higher leaf count compared to cattle manure alone, and a 7% increase when combined with 50N+25P fertilization and biofertilization with *R. intraradices*. These findings emphasize the effectiveness of combining mycorrhizal fungi with cattle manure in the substrate. In *Robusta coffee*, an average increase of 15 leaves was reported when grown with cattle manure and *R. intraradices* (Ibarra-Puón *et al.*, 2014). Similar results were observed in cacao (Aguirre-Medina *et al.*, 2007) and oro azteca coffee (Aguirre-Medina *et al.*, 2011). In other crops, mycorrhizal fungi have also been associated with similar leaf numbers in biofertilized plants, as seen in *Acacia farnesiana* and *Prosopis glandulosa* grown in substrates such as tepetate, forest soil, and agricultural soil (Hernández-Martínez *et al.*, 2006).

Physiological components

Root biomass accumulation in *Swietenia humilis* (caobilla) exhibited significant variations among treatments (Figure 1).

Root dry matter allocation in *Swietenia humilis* (caobilla) increased across all treatments compared to the control, with statistically significant differences ($P \leq 0.05$). The highest increase was observed in the treatment with 100_N+50_P fertilization and in the combination of cattle manure with seed biofertilization using *R. intraradices*. This response may be associated with nutrient availability provided by the cattle manure and high chemical fertilization, as other studies, such as those in cacao, have shown reduced root biomass allocation when plants are biofertilized with *R. intraradices* (Aguirre-Medina *et al.*, 2007). Additionally, the greater root development observed with the *R. intraradices* treatment could be linked to an increase in growth regulators induced by the symbiotic interaction. Aguirre-Medina *et al.* (2011) reported enhanced root system dry weight in *Coffea arabica* after inoculation with *R. intraradices*. Rovira *et al.* (1983) also emphasized the importance of root growth stimulation by microorganisms as a major contribution of symbionts to plant development. Once root colonization is established, plants show an improved capacity for nutrient uptake and distribution, enhancing overall nutrition (Aguirre-Medina and Kohashi, 2002). Similar responses have been observed in other

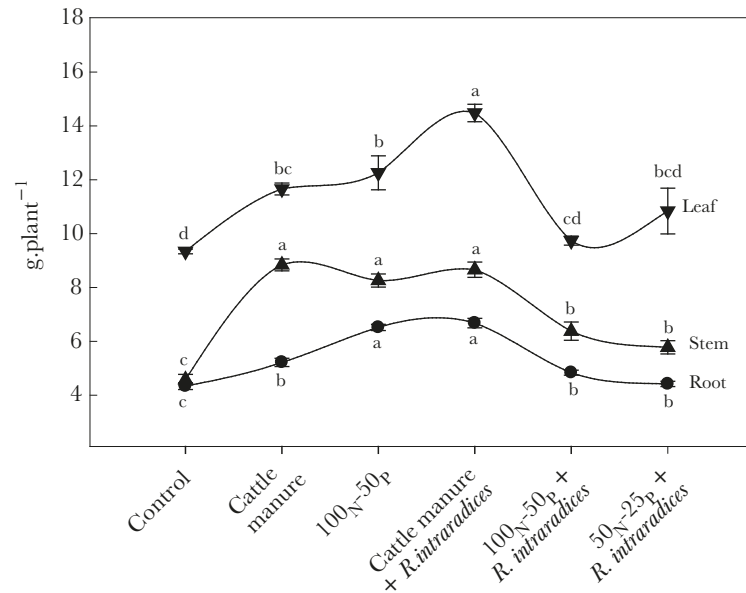


Figure 1. Dry weight of physiological components of *Swietenia humilis* Zucc. biofertilized with *Rhizophagus intraradices* in interaction with cattle manure and chemical fertilization. Values represent means of five replicates \pm standard error, and different letters indicate statistical differences (Tukey $P \leq 0.05$). CV=Root 55%, Stem 6.1%, Leaf 89.1%.

forest species, such as *Swietenia mahagoni* L. Jacq., where endomycorrhizal association led to greater root and shoot biomass accumulation (Falcón *et al.*, 2021). In terms of stem dry weight, all treatments showed increases compared to the control, with the highest values observed in treatments that included cattle manure, with or without *R. intraradices*, and in the 100_N+50_P fertilization treatment. These treatments formed the top statistical group, with significant differences from the remaining treatments ($P \leq 0.05$). The increased biomass allocation to the stem under these conditions suggests a differential partitioning of photosynthates, possibly influenced by changes in the rhizosphere microbial community due to root exudates (Linderman, 1988). The mycorrhizal association allows plants to allocate fewer carbohydrates to root system maintenance, favoring above-ground growth (Pereira *et al.*, 2001). The positive effect on stem thickness has also been documented in various crops. Aguirre-Medina *et al.* (2011) reported increased stem thickness in the coffee variety Oro Azteca following biofertilization with *R. intraradices* in the nursery stage. Similar results were observed in Marsellesa, Geisha, Sarchimor, and Costa Rica 95 coffee varieties (Anzueto-Herón *et al.*, 2023) and in species such as *Acacia farnesiana* and *Prosopis glandulosa* inoculated with mycorrhizal fungi (Hernández-Martínez *et al.*, 2006). Manjarrez-Martínez *et al.* (2000) noted that morphological and physiological responses of the host plant depend on the availability of carbon and phosphorus in the soil. Leaf dry weight (including lamina and petiole) also increased significantly in the treatment with cattle manure combined with *R. intraradices*, showing the highest biomass accumulation at 140 DAT and statistically outperforming the other treatments. In *S. mahagoni* L. Jacq., biofertilization with *Glomus cubense* and *Rhizophagus irregularis* similarly improved seedling growth and nutrition (Falcón *et al.*, 2021). These results support the variable functionality

of *R. intraradices* in plant interactions, which can induce physiological changes in the host plant (Jäderlund *et al.*, 2008; Barea *et al.*, 2002), as observed in *Leucaena leucocephala* (Aguirre-Medina *et al.*, 2018). The differential biomass allocation among caobilla growth components may also be influenced by root exudates, which facilitate colonization by other microorganisms affecting root morphogenesis and nutrient uptake (Berrabah *et al.*, 2018). In this study, the significant biomass accumulation observed with *R. intraradices* and cattle manure suggests a functional affinity between the fungus and the host plant, potentially supported by sufficient carbon allocation from the plant to the mycorrhizal partner. This finding confirms the differential growth induction in caobilla seedlings in response to the various treatments applied at sowing.

Root-to-shoot ratio

The root-to-shoot ratio exhibited contrasting changes among treatments, whether applied individually or in combination. The increase in the root-to-shoot ratio appears to be associated with the modular growth pattern of the plants. A notable increase in root biomass was observed with the 100_N+50_P fertilization treatment, while the highest shoot biomass was recorded when seeds were biofertilized with *R. intraradices* and cattle manure was incorporated into the substrate. In contrast, treatments that combined fertilization with biofertilization using *R. intraradices* showed a reduction in both root and shoot biomass (Figure 2).

The differences observed in biomass allocation between shoot and root components indicate an interaction between the plant and microorganisms (Olanrewaju *et al.*, 2019). In the case of *R. intraradices*, the benefits to both aerial and root organs are linked to improved

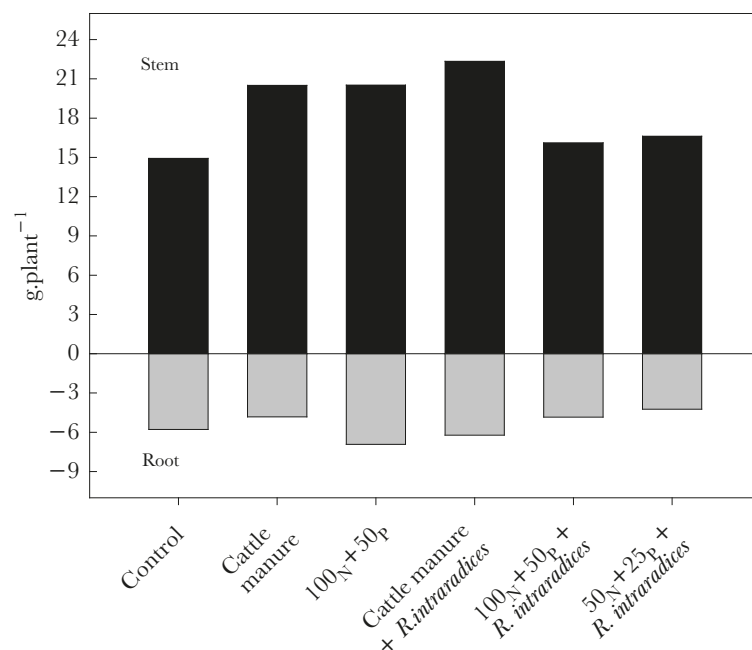


Figure 2. Root-to-shoot ratio of *Swietenia humilis* Zucc. biofertilized with *Rhizophagus intraradices* in interaction with cattle manure and chemical fertilization.

nutrient and water transport (Pereira *et al.*, 2001). This enhancement is facilitated by the hyphae of the fungus, which are thinner than plant roots and root hairs, allowing them to access soil zones that roots cannot penetrate, thus expanding the effective soil volume explored for nutrient acquisition (Ness and Vlek, 2000; He *et al.*, 2020). According to Perreta and Vegetti (2005), plant growth is regulated by genetic traits that vary within a specific range of phenotypic plasticity. The expression of modular growth across the plant's strata physiologically integrates the modules into a cohesive whole (Collado-Vides, 1997). In general, biofertilization of *Coffea arabica* L. with *R. intraradices* and *Azospirillum brasilense* significantly increases dry matter allocation across various yield components compared to control treatments (Vosatka and Albrechtova, 2009; Cogo *et al.*, 2017). In some cases, specific growth components may be more prominently expressed depending on the fungal isolate or when plants are biofertilized with microbial consortia (Vallejos-Torres *et al.*, 2019). Reduced growth induction in certain plants may be attributed to environmental and soil conditions that influence biomass accumulation responses. Endomycorrhizal fungi from one soil type may behave differently compared to those from other soils (Hart and Reader, 2002). This variability is also highly influenced by several environmental factors, including climatic conditions, as well as the age and variety of the host plant (Jefwa *et al.*, 2004).

CONCLUSIONS

Biofertilization with *Rhizophagus intraradices*, combined with the addition of cattle manure to the substrate and the application of 100_N+50_P chemical fertilization, promoted an increase in the biomass of morphological and physiological yield components of *Swietenia humilis* Zucc. compared to the control. The most notable differences in the morphological components of caobilla were observed from 56 days after transplanting (DAT). Both shoot and root biomass in caobilla exhibited differential responses depending on the interaction between cattle manure, *R. intraradices*, and chemical fertilization.

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