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RESEARCH ARTICLE

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Effect of arbuscular mycorrhizae on the growth of *Cinchona officinalis* L. (rubiaceae) in nursery

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ABSTRACT

Cinchona officinalis, commonly called cascarilla or quina, has medicinal value; and is on Peru's national coat of arms representing its plant wealth (flora), however, it is threatened by anthropogenic activities. This study aimed to determine the effect of the commercial product Myco Grow® on the growth of *C. officinalis* in nursery. A randomized design was used with two treatments, one with Myco Grow® application (WM) and the other without incorporating this commercial product (NM). Each treatment had three replicates consisting of 30 plants each. Monthly evaluations were performed, during which the number of dead plants, plant height, and plant diameter were recorded. Additionally, at the end of the study, the anhydrous weight of leaves, stems, and roots; leaf area; mycorrhizal frequency; mycorrhizal colonization index; and the length of extra-radicular mycelia were determined. The WM treatment achieved 36.6% lower mortality, 38.01% greater height, and 48.52% greater diameter than the NM treatment. Additionally, inoculation with arbuscular mycorrhizae (AM) improved the anhydrous weights of the leaves, stems, roots, and leaf area by 84.31%, 84.28%, 70.85%, and 76.91%, respectively. Regarding the three fungal variables analyzed for the WM treatment; mycorrhizal frequency was 87%, AM application led to a mycorrhizal intensity of 7.7% and an extra-radicular mycelium length of 90.3 cm. This study confirmed that AM positively influences the growth of *C. officinalis* in the nursery and can be used to sustainably produce this species on a large scale.

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Introduction

Cinchona officinalis L. is a forest species whose bark contains alkaloids that have medicinal importance. This has been used for centuries as one of the main treatments for malaria until the 1940s (Cóndor et al. 2009; Bharadwaj et al. 2018). Cinchona alkaloids are considered to be the most influential tree bark-derived medicines in human history (Prendergast and Dolley 2001); moreover, this species is found on Peru's national coat of arms representing its plant wealth (Flora) (García et al. 2022).

Cinchona officinalis requires special conditions to grow and its distribution is limited (Armijos-González and Pérez-Ruiz 2016). As of 2022, expanding agricultural and livestock land have degraded the *C. officinalis* habitat (Huamán et al. 2019; Fernandez et al. 2022). Additionally, this species has slow growth at the nursery level which is affected by edaphoclimatic (Fernandez et al. 2021) and microbiological (mycorrhizae) characteristics (Fernandez-Zarate et al. 2022).

Associations between plant roots and mycorrhizal fungi that can influence plant growth have been previously reported (Martínez and Pugnaire 2009). Arbuscular mycorrhizas (AM) adhere to the root system, forming extra-root hyphae that allow greater root elongation and, consequently, plants to absorb additional nutrients and water, thus improving their growth and development (Mehmood et al. 2022), in addition, fungal structures are directly related to the absorption of phosphate, ammonium, nitrate and amino acids by plants (Parniske 2008).

Mycorrhizal associations in forest nurseries have been studied in economically important forest species, such as pines, oaks, and spruces (Iwański et al. 2006; Menkis and Vasaitis 2011; Pietras et al. 2013; Rudawska et al.

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2017). However, studies on this type of symbiotic association in native tree species used in various local restoration and recovery strategies are scarce (Timonen and Kauppinen 2008; Stanturf et al. 2014; Rudawska et al. 2017). Therefore, generating information on *C. officinalis* behavior at the nursery stage to initiate plans for its management and preservation is necessary (Fernandez et al. 2022). Thus, this study aimed to determine whether applying AM to the substrate influences the growth of *C. officinalis* in the nursery stage. Additionally, AM could be used as a bio-fertilizer and biostimulant to accelerate *C. officinalis* growth in the future.

Materials and methods

Study area

The research was carried out in the La Cascarilla locality (5° 40′ 21.12″ S and 78° 53′ 55.65″ W), province of Jaén, Peru, which is located at 1810 m altitude, with an annual rainfall of 1730 mm, and an average temperature of 16.5 °C (Fernandez et al. 2021).

Plant material

For producing *C. officinalis* plants, seeds collected were used from a single population of *C. officinalis* from the community of San Luis (6°22′ 6.68″ S and 79°3′ 29.50″ E) at 2489 m altitude. Mature capsules (1 kg, brown to dark brown) were collected and transported in cloth bags to the La Cascarilla community, where they were stored in the shade. Seeds without visible cracks and/or contaminated by fungi were selected 20 d later for use in this study (Fernandez-Zarate et al. 2022). Seed storage was avoided because they rapidly lose their germination capacity owing to their recalcitrant nature (Caraguay et al. 2016). A subirrigation chamber was used for geminating *C. officinalis* seeds as described by Fernandez et al. (2021). Seeds were sown in a forest substrate, whose textural class was sandy loam, pH = 3.87, electrical conductivity = 0.15 dS m^{-1}, phosphorus = 9.95 ppm, potassium = 187.42 ppm, nitrogen = 9.53%, organic matter = 10.55% (Fernandez et al. 2022), autoclaved at 105 °C for 1 h, and this process was repeated for three consecutive days. *C. officinalis* seedlings were transplanted into 280 WM^3 cylindrical polyethylene bags (5.8 diameter and 10.6 height).

Microbiological inoculation

An inoculum of *Rhizophagus irregularis, Funneliformis mosseae* and *Glomus aggregatum* contained in the commercial product MycoGrow® – Complex was used. Six kilograms of this product were applied to one cubic meter of substrate, then uniformly mixed and placed in polyethylene bags.

Substrate

For the transplanting of *C. officinalis*, a substrate was prepared based on forest soil and sand (2:1), which was subjected to a sterilization process in an oven at 105 °C for 1 h. This process was replicated for three consecutive days. The physical and chemical properties of the substrate were as follows: sandy loam texture, pH = 4.18, electrical conductivity = 0.51 dS m^{-1}, organic matter = 6.03%, total nitrogen = 0.3%, and phosphorus = 8.5 ppm.

Experimental design and set-up

Two treatments were used, one with arbuscular mycorrhizae (WM) and one without mycorrhizae (NM), and three replicates per treatment. Thirty *C. officinalis* plants were used per replicate and 180 plants were used for the entire investigation. The *C. officinalis* seedlings were placed in polyethylene bags and monitored every month for 10 months.

The effect of AM on *C. officinalis* growth in the nursery was estimated by recording the number of dead plants, plant height, and plant diameter (measured using a digital Vernier at the substrate level). Additionally, the weight of leaves, stems, roots, leaf area, and fungal variables (mycorrhizal frequency, mycorrhizal intensity, and length of extraradicular mycelia) were calculated at the end of the trial.

The dry matter of the leaves, stems, and roots was determined by drying in an oven at 60 °C for 72 h, and the weight of each plant part was subsequently obtained.

The leaf area (cm^2) was calculated by taking photographs of leaves on a blank background (20 × 12 cm cardboard) that had a 2 cm long line drawn next to the leaf location site necessary to maintain the scale in image processing. The leaves were prevented from wrinkling by covering them with transparent glass (20 × 12 cm and 2 mm thick) and were photographed using a Huawei cell phone with a camera model MAR-LX3A of 24 megapixels. Images were processed using ImageJ (Baker et al. 1996).

Finally, mycorrhizal frequency (MF) (Sieverding et al. 1991), mycorrhizal intensity (MI) (Trouvelot et al. 1986) and length of extraradical mycelium (LEM) (Newman 1966; Carballar 2010) were determined.

Data analysis

The means of the replicates of each treatment were compared using a *t*-test ($\alpha = 0.05$) after testing for compliance with the normality assumption (Shapiro–Wilk) and homogeneity of variance (Levene's test). The results were analyzed using StatGraphics Centurion XVI (StatPoint Technologies Inc., Warrenton, VA, USA. JU).

Results

After 44 d of the *C. officinalis* transplant, the percentage of accumulated mortality in both treatments differed, reaching 86.6% dead plants at the end of the trial for the NM treatment, which was 36.6% higher than that in the AM treatment (Figure 1A). The cumulative increase in height at the end of the WM

treatment was 38.01% higher than that of the NM treatment (Figure 1B). A similar result trend was obtained for the increase in diameter, which was 48.52% higher in the WM treatment than that in the NM treatment (Figure 1C). This indicated that AM had a significant and positive influence on the growth of *C. officinalis* at the nursery stage. Generally, *C. officinalis* plants inoculated with AM showed a lower mortality rate and reached greater heights and diameters than those without AM treatment.

AM inoculation resulted in a significantly higher dry biomass yield of leaves (Figure 2A), stems (Figure 2B), and roots (Figure 2C) than when no AM was inoculated. Furthermore, the leaf area of *C. officinalis* plants in the WM treatment was significantly higher than that in the NM treatment (Figure 2D), representing an increase of 79.91%.

Regarding the three fungal variables analyzed for the WM treatment, the results are shown in Table 1.

Discussion

AM application led to a 36.6% reduction in *C. officinalis* plant mortality at the nursery stage compared with NM treatment, this result could be associated with the positive effects of AM in protection against pathogens, leading to early plant death (Berruti et al. 2015; Devi et al. 2022; Dey and Ghosh 2022). Additionally, a significant increase in the height and diameter of *C. officinalis* was observed in the WM treatment, these results could be directly related to the production of more efficient root systems, since plants inoculated with AM had significantly higher root biomass than plants that were not inoculated with AM (Quoreshi and Khasa 2008). AM colonizing roots provides better nutrition (Pankaj et al. 2021) because mycorrhizae can increase root size by up to 200% (Falcón et al. 2021), allowing greater access to nutrients in the soil (Weisany et al. 2016; Khalediyan et al. 2021). Several studies have suggested that AM generates growth hormones such as auxins and indole acetic acid, which influence plant development, physiology, morphogenesis, and root growth (Quoreshi and Khasa 2008; Smith and Read 2008), this proves the potential benefits to be obtained with AM inoculation on *C. officinalis* plants at the nursery stage.

The anhydrous weights of leaves, stems, and roots were higher in plants inoculated with AM than in those without AM, suggesting that the binding between AM and the roots of *C. officinalis* improves nutrient uptake by the plant, as demonstrated for a *Theobroma cacao* crop, and water uptake (Jiang et al. 2017; Aggangan et al. 2019).

Additionally, the present study revealed an increase in leaf area in the WM treatment, which could be attributed to the percentage of mycorrhizal colonization (Palacios et al. 2021; Fernandez-Zarate et al. 2022) and the length of the extraradicular mycelium, which was higher in plants inoculated with AM. AM colonization on *C. officinalis* roots can lead to increased uptake of water and nutrients, as it increases the surface area through the mycelium in the soil, providing the plant with greater access to the soil. This can lead to increased photosynthetic efficiency, improved plant growth, and, subsequently, increased leaf area (Huang et al. 2018; Khalediyan et al. 2021).

Conclusion

AM inoculation decreased in mortality and increased in height, biomass and diameter of *C. officinalis* plants, and improves the evaluated fungal parameters (MF,

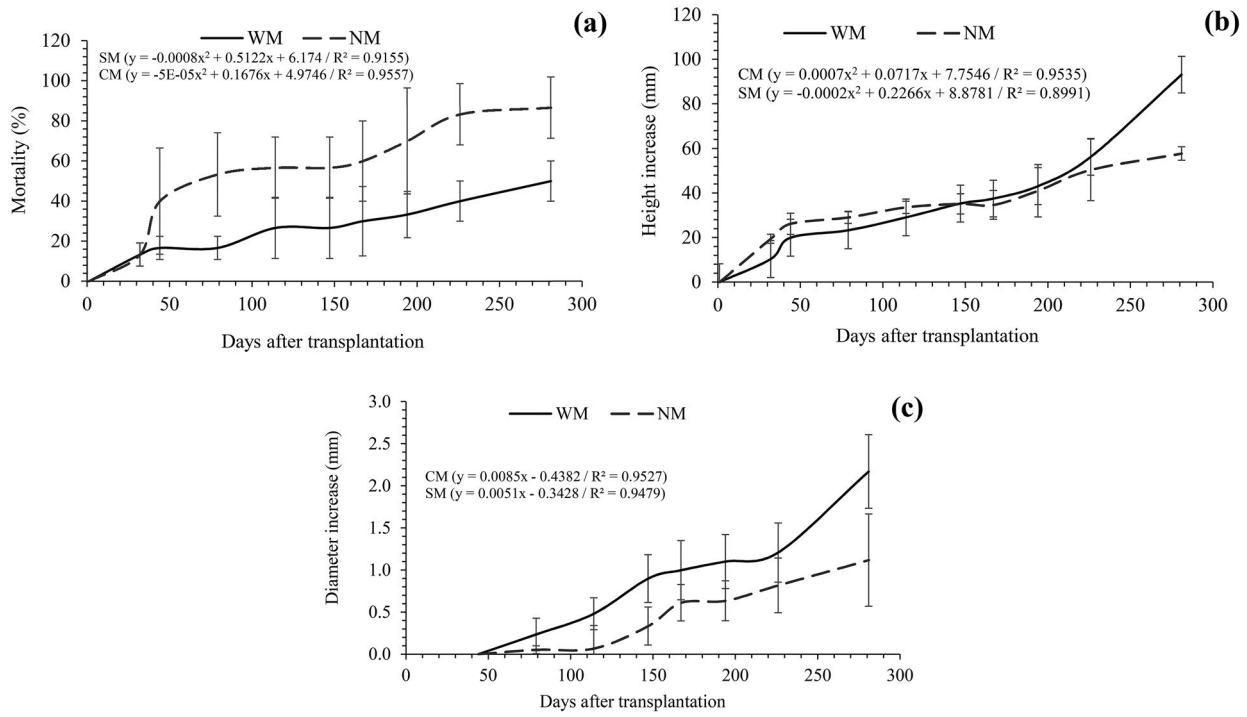

Figure 1. Cumulative percent mortality of *C. officinalis* during the entire trial (a), height increment (B), and diameter (C) of *C. officinalis*.

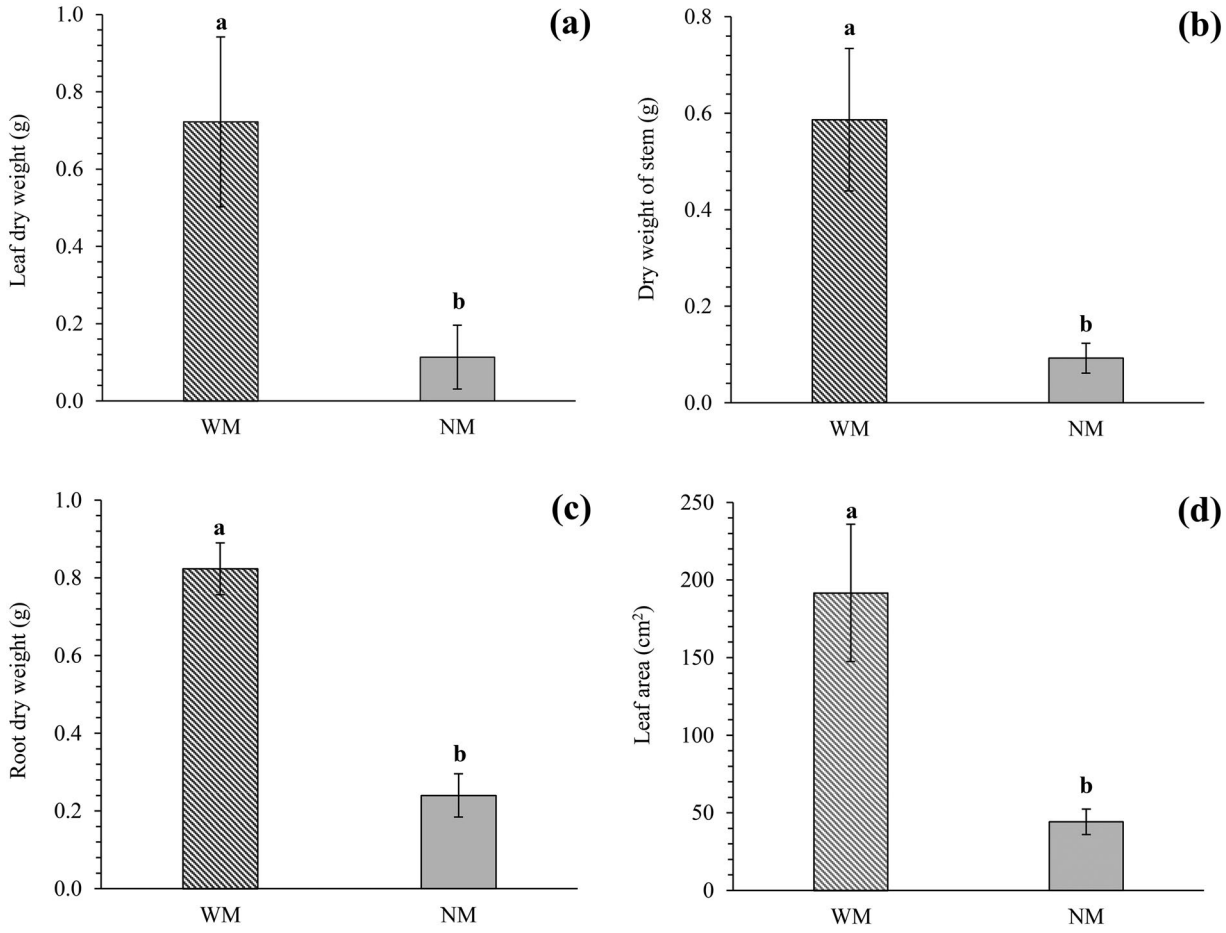

Figure 2. The evaluated growth parameters. Anhydrous leaf weight (a), anhydrous stem weight (B), anhydrous root weight (C), and leaf area (D). Different lowercase letters indicate significant statistical differences ($p = .05$).

Table 1. Fungal parameters analyzed for the WM treatment.

Fungal parameter	Mean ± SD
Mycorrhizal frequency (%)	87.00 ± 1.15
Mycorrhizal intensity (%)	07.70 ± 1.01
Length of extraradicular mycelium (cm)	90.30 ± 10.10

MI, and LEM). This suggests that AM can be used as a biofertilizer for *C. officinalis* at the nursery stage. This study did not analyze the effect of a specific mycorrhiza, but evaluated a mycorrhizal consortium formed by *Rhizophagus intraradices, Funneliformis mosseae,* and *Glomus aggregatum*, in this sense, if the effect of each species on the growth of *C. officinalis* is desired, each of the mycorrhizae should be analyzed independently. The results show promise for AM application in nurseries to produce *C. officinalis* plants and contribute to repopulation programs of the species.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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