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The effect of arbuscular mycorrhizal fungi on the growth of cyclocarya paliurus cutting seedlings

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Abstract: To increase the yield of secondary metabolites, Arbuscular mycorrhiza fungi were used to improve cyclocarya paliurus. The experiment measured growth indicators, biomass, mineral nutrients, etc. The results showed that after inoculation with mycorrhizal fungi the nitrogen content in the leaves of the experimental group was 27.87 mg/g, K content was 7.48 mg/g, Ca content was 26.63 mg/g, and Mg content was 2.82 mg/g. Compared with the control group (Cg), mycorrhizal fungi can promote the absorption of mineral nutrients by *C. paliurus* seedlings. *A. mycorrhizal* fungi had a certain influence on the proportion of root, stem, leaf, and total biomass of *C. paliurus* seedlings, and their biomass distribution in various parts was relatively balanced. *A. mycorrhizal* fungi can significantly increase the biomass of willow seedlings' stems and significantly increase their proportion in the total biomass ($p < 0.05$). After mycorrhizal inoculation, the secondary metabolic yields of quinic acid, quercetin, and kaempferol in *C. paliurus* leaves were 0.87 mg/g, 0.32 mg/g, and 0.65 mg/g, respectively, higher than those in the Cg. It is concluded that the use of *A. mycorrhizal* to improve the survival rate of medicinal materials and ensure the quality of medicinal materials is a new idea for the industrial production of *C. paliurus*.

Key words: *Arbuscular mycorrhizal*, green willow, secondary metabolism, inoculation, content, yield

1. Introduction

Qing qian liu forest resources are scarce, mainly natural forests, and most of them are located in remote mountainous areas and nature reserves. Due to its limited natural resources, there is currently a growing demand for it, mainly through artificial farming. Cyclocarya paliurus is a woody material with Chinese medicinal characteristics. It is rich in secondary metabolites such as flavonoids and triterpenes and plays an important role in promoting human health. Green willow leaves also have significant effects on lowering blood lipids and cholesterol and treating coronary heart disease (Veresoglou et al., 2019). With the development and advancement of China's pharmaceutical industry, the demand for green willow resources is constantly increasing. However, due to the scarcity of such resources, the development of artificial forests has been a key aim of China's pharmaceutical industry. During this development period, a large amount of inorganic fertilizers was used. Fertilization not only promotes plant growth and increases yield (Norouzi et al., 2021; Khoshkhoo et al., 2022; Arji et al., 2022; Salehi-Sardoei et al., 2023) but also leads to the rapid synthesis of effective secondary metabolites (SMs) in plants (Almasi, 2021; Falaknaz et al., 2022; Jalilian et al., 2022; Kakaeian et al., 2022). Heavy fertilization has also had an inactive impact on the ecological environment. How to improve the yield and quality of *Cyclocarya* from other aspects is the direction of future development (Arji et al., 2021; Aryafar et al., 2021; Rahimian et al., 2021; Rostami Ahmadvandi and Faghihi, 2021). Mycorrhizal fungi (MF) refer to a mutualistic symbiosis formed by fungi in the rhizosphere soil and parasitic higher plant roots. MFMF can be mainly divided into three categories, one of which belongs to the main type of endophytic MF. The most extensively distributed kind of MF in this type is the vesicular *Arbuscular mycorrhizal*, which is the undivided endophytic MF (Rondina et al., 2019). At the root of the host plant, there are a large number of extracellular cells. Some fungi do not have an extracellular cell membrane; hence they are called *A. mycorrhizal*. *A. mycorrhizal* is an important bacterial species widely distributed in nearly 90% of higher plants, and can directly or indirectly participate in the material exchange between plants and soil. *A. mycorrhizal* mycorrhiza is in a ubiquitous symbiotic relationship with plant roots that can facilitate the growth of plants and give an essential material foundation for the accumulation of

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SMs in plants. *A. mycorrhizal* can have an impact on the secondary metabolic activities of plants (Zhurbenko et al., 2020). With the widespread application of *A. mycorrhizal* in forests, utilizing *A. mycorrhizal* to improve the survival rate of medicinal materials and ensure their quality has provided new ideas for the industrial production of *C. paliurus*. To reduce the amount of chemical fertilizer and balance the contradiction between the yield and quality of *C. paliurus* plantation, in the present study the aim was to promote the growth of *C. paliurus* plantations and increase the production of its SMs by inoculating *A. mycorrhizal*. It was also aimed to elucidate the MF infection impact on the growth characteristics, biomass accumulation, C and N content, mineral nutrient absorption, SM content, and yield of cyclocarya paliurus seedlings after MF infection at different growth stages. This paper is mainly divided into five sections. The first part is the introduction, which briefly introduces the research background. The second part is a literature review, summarizing the relevant research of scholars in different fields and pointing out the purpose of the present study. The third part contains the experimental design. The fourth part is the result analysis, which analyzes the impact of inoculation with MF on the growth, secondary metabolism, and other aspects of *C. paliurus* seedlings. The fifth part is the conclusion and also points out the direction of future research.

A. mycorrhizal can promote the utilization of nutrients in the soil by host plants. The research on *A. mycorrhizal* and its application in forests has attracted widespread attention, and scholars in various fields have conducted extensive research and achieved good research results (Salehi-Sardoei A et al., 2022).

To study the effect of fungi on the growth of willow cuttings, scholars such as Jean et al. (2021) increased the stem biomass of cuttings by growing rhizopus on sterile waste rocks. Research has found that *Melonomyces* sp. has an active effect on unsterilized waste rock cuttings, while *P. fortinii* strain does not influence its survival rate, aboveground yield, or biomass yield (Jean et al., 2021). Stolarski et al. (2019) cultivated three different willow varieties on three different types of soil in order to measure the yield of willow trees cultivated in different types of soil, and used limited fertilization and cultivation measures. The experiment showed that willows can be cultivated on a large scale in the Eko-Salix system, and yield is closely related to varieties. The biomass on peat soil and humus alluvial soil is obviously larger than that on clay (Stolarski et al., 2019). Kokkoris et al. (2020) compared the important driving factors of *A. mycorrhizal* communities in plants and evaluated the spatiotemporal changes in host preferences and the classification resolution of plants and *A. mycorrhizal* to study the causal relationship between plant and *A. mycorrhizal* symbiosis. Host identity can only explain less than 30% of the variation in *A. mycorrhizal* groups, and plant *A. mycorrhizal* pairing has functional elements for symbiosis (Kokkoris et al., 2020). Wang et al. (2021) conducted research on how *A. mycorrhizal* responds to plant immune system issues and found that RiNLE1 can be transported to the host cell nucleus, weakening the mononucleosis of H_2B and enhancing the level of inhibition and colonization of defense gene expression. Moreover, *A. mycorrhizal* can inhibit the genetic modification of the plant landscape in combination with the direct interaction of core nucleosome components, indicating that RiNLE1 class effectors can be used to manipulate the host's defense response (Wang et al., 2021). Standish et al. (2021) conducted experiments to investigate the impact of underground interaction on community dynamics, and investigated the impact of *A. mycorrhizal* nuclear phosphorus supply gradient on the competition and role of microscopic woody plants. Plants have different competitive effects under low and high phosphorus supply. In the highest phosphorus state, the beneficial effects of *A. mycorrhizal* inoculation on mycorrhizal species are greatly reduced (Standish et al., 2021).

Wang et al. (2021) discussed the effect of rhizobia and *A. mycorrhizal* inoculation on cadmium resistance of purple flowers in order to study the mechanism of microbial influence on the rhizosphere microbial community and its change in plant resistance to metal stress. Inoculation of rhizobia or *A. mycorrhizal* alone will help to improve the resistance of purple flowers to cadmium stress, and the effect of inoculation of both together will be better (Wang et al., 2021). Dueas et al. (2020) proposed a study on the impact of increased phosphorus on soil biodiversity, utilizing full factor nitrogen and phosphorus on the composition of mixed *A. mycorrhizal* communities. When nitrogen and phosphorus are added together, the changes in abundance can be observed and analyzed. When the concentration increases moderately, it will have a long-term impact on the tropical *A. mycorrhizal* community (Dueas et al., 2020). To study the role of soil invertebrates in *A. mycorrhizal* development, Chen et al. (2022) analyzed the chemical signal pathway between invertebrate nuclear *A. mycorrhizal* fungi and provided a new method for promoting the research of ecosystem elasticity and sustainability (Chen et al., 2022). To understand the nutrient exchange relationship between host plants and *A. mycorrhizal*, Wipf et al. (2019) studied the transfer of nitrogen and phosphorus from host plants by fungi and analyzed the interaction between nitrogen and phosphorus nutrients during *A. mycorrhizal* symbiosis. The study ultimately obtained the common mycorrhizal network structure formed by *A. mycorrhizal* (Wipf et al., 2019). Chaudhary et al. (2020) proposed a trait-based method for predicting the airborne spread of *A. mycorrhizal* fungi in

order to study the different species-based predictions in this airborne spread. They conducted one-year *A. mycorrhizal* collection in the air at a height of 20 m and measured the morphological and functional characteristics of spores. Spores will promote air transmission and the diversity of *A. mycorrhizal* is high. The abundance and community structure of spores will undergo different changes over time (Chaudhary et al., 2020).

The above research indicates that *A. mycorrhizal* is a type of symbiotic organism widely distributed in nature, with strong adaptability to various ecological environments, and can promote the accumulation of SM in plants. Due to the use of fertilizers in the cultivation of *C. paliurus*, the SM content in the plant is greatly reduced, which has a negative impact on the quality of raw materials. Therefore, in the present study, *A. mycorrhizal* will be used to improve the growth of *C. paliurus* and promote secondary metabolism.

2. Materials and methods

In the experiment on the influence of *A. mycorrhizal* on growing green willow cuttings, it is necessary to first collect plant materials and perform relevant treatments, and design the experiment based on the research purpose. After that, relevant indicators need to be measured, including growth indicators and biomass, mineral nutrients of *C. paliurus* and each part, C and N content of *C. paliurus*, jasmonic acid, SM phenolic acid, SM triterpenes, etc. Finally, the yield of SM from *C. paliurus* is calculated.

2.1. Test materials and experimental design

Seeds of *C. paliurus* in a natural reserve were collected in October 2017 and methods such as acid etching and gibberellin soaking were used to break their dormancy. Then the seeds were freeze-thawed and sterilized in moist soil to germinate. In March of the following year, the exposed green willow sprouts were transplanted into a culture medium disinfected in a high-temperature sterilization tank, which can be used as plant materials for subsequent experiments (Okiobe et al., 2019). The research plan was to select a green willow tree with a good growth period in a nearby forest farm in late June of the following year, excavate fine roots from its surface layer, and dig out some of its rhizomes and surrounding soil, and bring them back for laboratory inoculation. Two treatments were set up in the experiment, with three replicates, resulting in a total of 10 green willow seedlings. The experiment used nondisinfected inoculation soil in the experimental group (Eg) and disinfected inoculation soil in the control group (Cg) (Rillig et al., 2020). In early July of the following year, green willow seedlings with similar growth conditions were transplanted into the test container, totaling 60 pots. The above experimental treatment adopted traditional management methods and irrigation once a week after mycorrhizal infection. All the green willow seedlings were placed in an artificial climate box with a light intensity of 380 μmol/m^2/s, with a light duration of 12 h, day and night temperatures of 25 °C and 15 °C respectively, and relative humidity of 65% in the air (Nakamura et al., 2020).

2.2. Measurement indicators and methods

The measurement indicators and methods mainly included observation of *A. mycorrhizal* infection, determination of growth indicators and biomass, mineral nutrients (K, Ca, Mg, P) of *C. paliurus* and each part, the C and N content of *C. paliurus*, jasmonic acid, SM of phenolic acid, flavonoids, and triterpenes, and calculation of the SM output of *C. paliurus*, as shown in Figure 1.

The observation of *A. mycorrhizal* infection was conducted by collecting fine roots and roots of *C. paliurus* seedlings from the ancient woodland of *C. paliurus* to stain and observe the infection of MF. Firstly, a small amount of white to light yellow-green willow seedling roots was washed and filtered, then classified and labeled, placed in liquid nitrogen, and finally stored in a –70 °C ultralow temperature freezer for later use. Afterward, the root system was decolorized and cut into small segments of approximately 1–2 cm. The segments were placed in a numbered 10-mL centrifuge tube, followed by addition of 1 mL of 20% KOH solution, and the tube was put in a 90 °C water bath for 30 min. After thoroughly removing the cytoplasm of the root cortical cells, they were rinsed with tap water 3–5 times, then checked for the water content, and left to stand (Dou et al., 2019). At room temperature, a small amount of alkaline H_2O_2 was added, decolorization was maintained for 2 h, and then they were rinsed repeatedly with distilled water 3–5 times to control drying. Next, 1 mL of 5% glacial acetic acid was added and they were acidified at room temperature for 5 min. The next step was to perform postdyeing decolorization treatment: 95 mL of 5% concentration of glacial acetic acid was mixed with 5 mL of Parker brand blue black ink to produce a 5% concentration dye. Two drops of the staining agent were dropped into a centrifuge tube and the decolorized and softened roots were dyed and numbered separately. Then they were heated in a 66 °C water bath for 30 min, rinsed 5 or 6 times with distilled water, and finally soaked in clean water for more than 12 h to complete the decolorization. Finally, preparation and observation were carried out: the decolorized root segments were dried with absorbent paper and placed on a glass slide. Three preparation treatments were set up and performed 3 times (Fu et al., 2019). The experiment used a Zeiss electron microscope to observe, photograph, and record the samples.

For the determination of growth indicators, the height of the marked plants was measured with a steel tape and the ground diameter was measured with a Vernier scale. After measuring the growth indicators, a portable

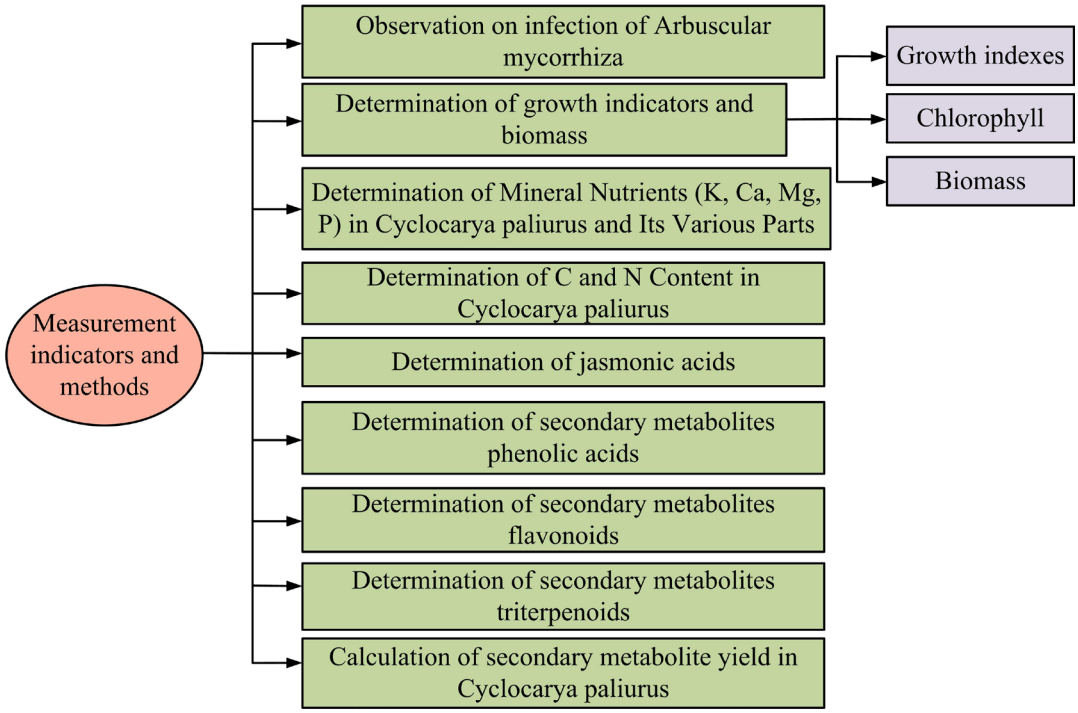

Figure 1. Measurement indicators and methods.

chlorophyll analyzer was used to measure the relative chlorophyll content of the leaves of *C. paliurus* seedlings. In the last month, when the height and diameter of the green willow seedlings had increased, they were dug out of the ground and divided into three parts: roots, stems, and leaves. They were washed, filtered, and placed in an 80 °C oven for drying. After the temperature dropped to a certain level, the biomass of each part of the green willow seedling was weighed and the total biomass was calculated, and it was ground into powder. Afterward, the mineral nutrients including K, Ca, Mg, and P were determined for the green willow and its various parts. First 0.5 g of plant sample powder was accurately weighed and digested with hydrogen chloride nitrate. A constant volume of 100 mL of boiling solution was prepared and 0.1 g of sample was added, followed by stirring and then boiling (Norman and McManus, 2019). The next step was to determine the C and N content of *C. paliurus*: 3.5–4 mg of the powder sample to be tested was weighed, the sample was rolled into a round ball with a tin cup, and an element analyzer was used to measure the C and N content of each organ part of the sample.

Then jasmonic acid was determined; the leaves were ground fully in liquid nitrogen and stirred evenly. The ground plant powder was stored in the refrigerator at –20 °C for standby. The sample to be tested was impregnated with methanol, and the impurities were removed by the solid phase extraction column. Then dihydro jasmonic acid was used as the internal standard for quantitative analysis with UPLC-MS/MS. Next, the secondary metabolite phenolic acids were determined: total flavonoids, quercetin, and kaempferol were determined by corresponding methods (Crue et al., 2019). In the production of the total flavonoid standard curve, under high-temperature drying, 10 mg of rutin standard sample was weighed, 80% ethanol solution was added, and it was dissolved, brought to a constant volume of 25 mL, and shaken well. Then 0 to 1.2 mL, every 0.2 gradients, was extracted for a total of 7 groups. It was placed in a 10-mL test tube, 10 mL ultrapure water was added, and it was shaken well and left to stand for 15 min. Its optical density in the 415 nm wavelength band was measured using a scanning ultraviolet-visible spectrophotometer. In the determination of total flavone content, 1 mL of extract was put into a 10-mL volumetric flask, 2 mL of ultrapure water was added and then 0.3 mL of 5% $NaNO_2$ solution, and it was left to stand at room temperature for 5 min. Then 0.3 mL of 10% aluminum chloride methanol solution was added and it was left to stand for 5 min. Next 1 mL of 1 mol/L NaOH solution was added and color developed at room temperature for 15 min. Constant volume was achieved with ultrapure water and the absorbance was measured at 415 nm. The total flavonoid content in the extraction solution was calculated and it was ultimately determined as milligrams of rutin

equivalent. All experiments were performed three times and the results obtained were taken as the average (Abdullah et al., 2022). In the determination of quercetin and kaempferol content, 1.0 g of *C. paliurus* sample was put into a conical bottle, then 25 mL of 25% hydrochloric acid was mixed in with methanol, and it was sealed and weighed. Then it was placed in boiling water and cycled for 60 min. After it had cooled naturally, it was weighed again, replenished with methanol and brought to volume, and then shaken evenly; this was performed twice. Finally, it was filtered with 0.45-μm organic film and used as the detection liquid for high-performance liquid chromatography.

Afterward, the determination of SM triterpenoids requires analysis of the content of total triterpenoids, arjunolic acid, and cyclocarya paliurus acid B. In the extraction of triterpenoids, 0.3 g of sample powder of each part of the root, stem, and leaf of *C. paliurus* was put into a conical flask, then 20 mL of 70% ethanol was added, and it was shaken and sealed. It was placed in an ultrasonic cleaner at 50 °C and 1000 W for 1 h, filtered with a filter paper funnel, and finally bottled. The determination of total triterpenoid content in the leaves of cyclocarya paliurus was based on the colorimetric method. reported by In the determination of the content of arjunolic acid and cyclocarya paliurus acid B, the content was confirmed using a high-performance liquid chromatography system, and the content of arjunolic acid was determined using the 2424 ELSD method. Finally, the yield of SM from *C. paliurus* was calculated. The experimental data were processed using Excel 2010, which summarized, organized, and analyzed the data, and conducted preliminary analysis and processing on the mapping. SPSS Statistics 24.0 with one-way ANOVA was used to compare mean values.

3. Results

We analyzed the effect of inoculation of mycorrhiza on *C. paliurus* seedlings, including the growth and yield of *C. paliurus* seedlings and the contents of N, C, C/N, K, Ca, Mg, P, jasmonic acid, and SM.

3.1. Analysis of the effect of inoculating MF on *C. paliurus* seedling growth

The *A. mycorrhizal* microscopic observation shows that the root mycorrhiza of *C. paliurus* is a kind of endomycorrhizal mycelium mainly composed of arbuscular MF, which is composed of an arbuscular structure, nonseptal hyphae, vesicles, etc. *A. mycorrhizal* is abundant in the root system of *C. paliurus*, and its mycelium can penetrate and interact with the epidermis of plants. Using the topsoil of *C. paliurus* ancient woodland, the seedlings of *C. paliurus* were inoculated at the beginning of July, and the height and ground diameter growth of the seedlings were recorded at the end of July to October. The Table shows the details.

According to the Table, in the first month after inoculation, the seedling height growth of each Tg was higher. However, from August to October, the plant height of the green willow seedlings in each Tg was significantly higher ($p < 0.05$). The growth trend of ground diameter and seedling height of the green willow seedlings is basically consistent, and all treatments are higher than the Cg. Therefore, the MF inoculation in the topsoil of *C. paliurus* ancient woodland significantly promoted the growth of seedling height and diameter at the seedling stage. The chlorophyll content can directly reflect the photosynthetic rate and nutritional status of plants. Nitrogen and magnesium are components of chlorophyll. Many mineral elements are also auxiliary factors of chlorophyll enzyme catalysis, and chlorophyll also has a direct impact on photosynthesis. The accumulation of plant biomass is the material basis for the medicinal production of *C. paliurus*. The size of plant biomass is affected by external factors, e.g., light, temperature, soil moisture, and nutrients, and it also reflects the ability of plants to absorb natural energy and adapt to external environments. This energy occupies a vital position in the growth and metabolism of plants. Figure 2 shows the difference in biomass of *C. paliurus* after inoculation with MF.

According to Figure 2, MF inoculation can significantly increase the total biomass of *C. paliurus* seedlings, and there

Table. *A. mycorrhizal* fungi inoculation effects and chlorophyll content of *C. paliurus* seedlings.

Index	Seedling height/cm		Ground diameter/mm		Chlorophyll/SPAD	
Handle	Unsterilized	Sterilized	Unsterilized	Sterilized	Unsterilized	Sterilized
July	16.33 ± 0.95 a	13.00 ± 2.37 a	1.99 ± 0.16 a	1.60 ± 0.17 a	27.24 ± 2.44 a	29.08 ± 2.10 a
August	26.14 ± 3.50 a	19.69 ± 4.44 b	2.69 ± 0.24 a	2.24 ± 0.01 a	28.96 ± 0.18 b	31.54 ± 1.65 a
September	44.05 ± 1.62 a	34.09 ± 7.76 b	4.43 ± 0.58 a	3.24 ± 0.40 b	31.54 ± 0.69 a	30.92 ± 0.79 a
October	57.85 ± 4.09 a	47.68 ± 7.30 b	5.69 ± 0.17 a	4.75 ± 0.40 b	35.79 ± 0.81 a	36.16 ± 1.19 a

Note: Minuscule indicates that there are significant distinctions among the treatments in the same month ($p < 0.05$); Experimental group = Eg, Control group = Cg. Different letters in each column indicate significant differences.

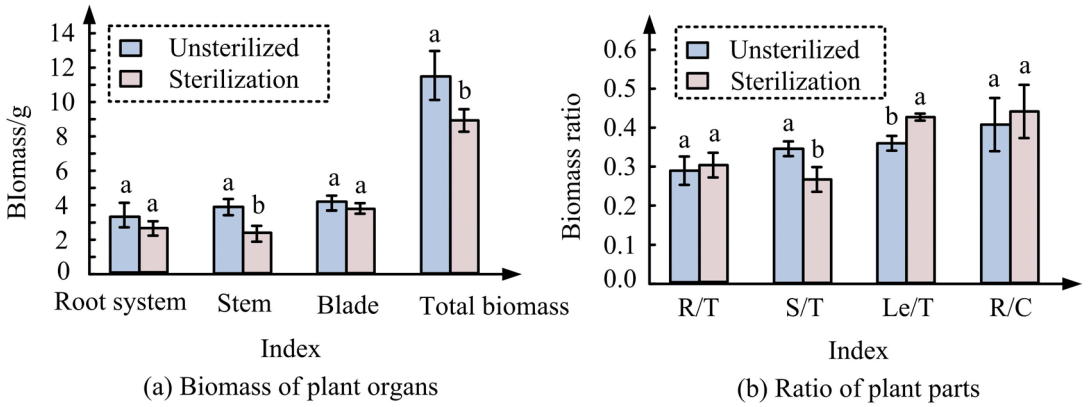

Figure 2. The effect of inoculating MF on the biomass accumulation of *C. paliurus* seedlings.

is a significant difference in biomass between *C. paliurus* seedlings after inoculation with MF ($p < 0.05$). The total biomass of the Eg was higher ($p < 0.05$), 29.3% higher. In terms of leaf and root biomass, the Eg had slightly higher leaf and root biomass than the Cg, while the stem biomass had an obvious increase compared to the Cg ($p < 0.05$). The proportion of biomass in each part of the green willow to the total biomass and the root crown ratio can better reflect the energy absorption and distribution of aboveground and underground substances in each part of the plant. According to Figure 2, mycorrhizal treatment had a certain impact on the biomass and root-to-shoot ratio of *C. paliurus* seedlings. The leaf-to-total biomass ratio was significantly lower than in the Cg, while the stem-to-total biomass ratio was higher ($p < 0.05$). The root cap was slightly lower. In the final month of the experiment, the main root, lateral root, and lateral root lengths of the entire plant of *C. paliurus* were measured, and the results are displayed in Figure 3.

As seen in Figure 3, the root length ranged from 13 cm to 29 cm. After inoculation with MF, there was no significant change or difference in lateral roots between the two groups. However, the unsterilized Eg was much higher than the sterilized Cg ($p < 0.05$). The average number of lateral roots in the unsterilized group was about 12 per plant, while the Cg had less than 9 per plant. Through analysis of variance, there was no significant difference in the quantity of lateral roots between the main and lateral roots of *C. paliurus* before and after inoculation with MF. However, the quantity of lateral roots in its seedlings compared to inoculation with MF was significantly different ($p < 0.05$).

3.2. Effects of mycorrhizal inoculation on N, C, and C/N of *C. paliurus* seedlings

C is an important component that forms plant dry matter, while N is a key nutrient element that affects plant growth and development. It is closely related to various physiological functions such as photosynthesis, cell division, and growth, but it is also a key factor that restricts plant growth and development. C/N is the main nutrient component of plants, accounting for over half of their dry weight. C has stress resistance and support functions, while N enhances plant stress resistance and support functions. An appropriate C/N ratio can significantly and effectively improve plant growth and development, and regulate plant growth. Figure 4 shows the effect of MF inoculation on N content.

The content of N in the leaves of cyclocarya paliurus in the unsterilized Eg and the sterilized Cg was 56.6 and 48.3 mg/g, respectively. The content of N in the roots of *C. paliurus* in the Eg and Cg was 17.89 and 16.8 mg/g, respectively. The nitrogen content in the stems of *C. paliurus* was 10.73 mg/g in the Eg and 8.6 mg/g in the Cg. The nitrogen content in the leaves of the Eg and the Cg was 27.87 and 22.89 mg/g, respectively. There was no significant difference in N element content in the roots, but the accumulation of N element in the stems, leaves, and total N element of *C. paliurus* seedlings did differ under different treatments. The stem, leaves, and total N element accumulation of the Eg were higher ($p < 0.05$). Both groups had the highest accumulation of N elements in the leaves, accounting for 49% and 47% of the total N content, respectively, which is higher than the 22% in the Cg. The stem content of both groups was higher than that of the Cg, while the N in the stems and leaves of the Cg was lower ($p < 0.05$). Its N accumulation is the lowest, which is 50% lower than that of leaves. The total amount of N in each component of the Eg was also higher. Figure 5 shows the effect of MF inoculation on C content.

As seen in Figure 5, C differs significantly between the unsterilized Eg and the sterilized Cg ($p < 0.05$). The C content in the three parts of plants for the two groups of treated *C. paliurus* was around 400 mg/g. A reasonable proportion of C and N is beneficial for growing plants and the accumulation of metabolites. Figure 6 shows the effect of MF inoculation on C/N content.

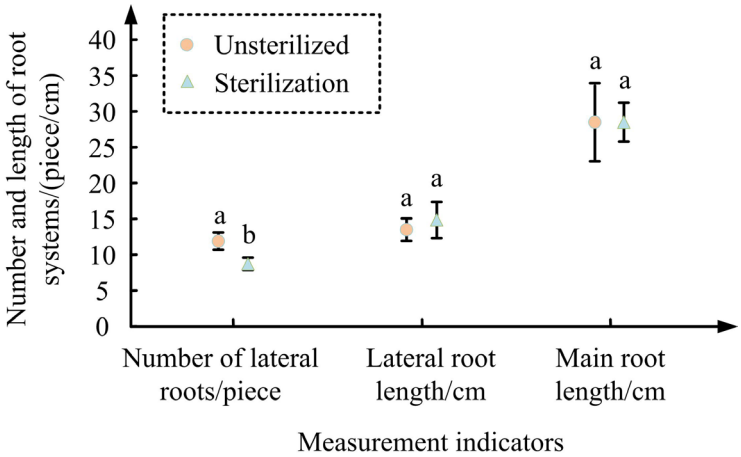

Figure 3. Inoculating MF on the root characteristics of *C. paliurus*.

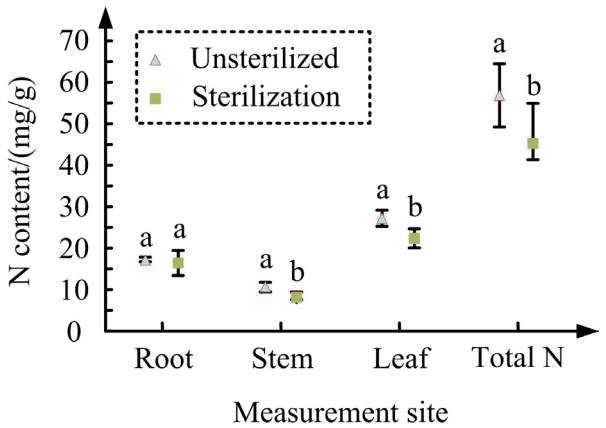

Figure 4. Inoculation with MF on N.

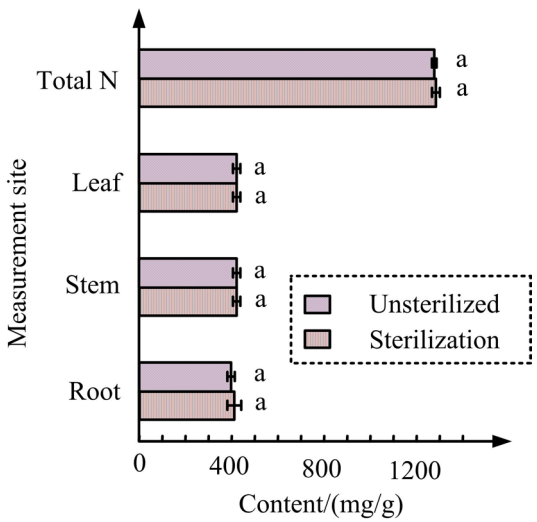

Figure 5. Effect of inoculation with mycorrhizal fungi on C content.

The overall traits of the unsterilized Eg were as follows: stem 50.12 > root 25.33 > leaf 18.86, while the overall traits of the Cg were: stem 40.11 > root 25.33 > leaf 15.58. The C/N ratio differed significantly between the two unsterilized groups ($p < 0.05$). The C/N of the stem in the unsterilized Eg was 40.1, while the C/N of the Cg was 50.1. According to the analysis of significant differences, the leaf ratio of the Cg was significantly higher ($p < 0.05$). There was also a significant distinction in the C/N content in the leaves of cyclocarya paliurus. The C/N content in the nonsterile Eg was 15.6, while the C/N content in the Cg was 18.9. In comparison, the two groups differed significantly ($p < 0.05$).

3.3. Effect of mycorrhizal inoculation on K, Ca, Mg, and P content in *C. paliurus*

Mineral nutrients have a crucial role in the growth and physiological metabolism of *C. paliurus*, and the effect of mycorrhizal inoculation on its K, Ca, Mg, and P content is exhibited in Figure 7.

From Figure 7, it can be seen that the content of K in the seedlings of *C. paliurus* is significantly different ($p < 0.05$) between the two treatments, and the total K content in the

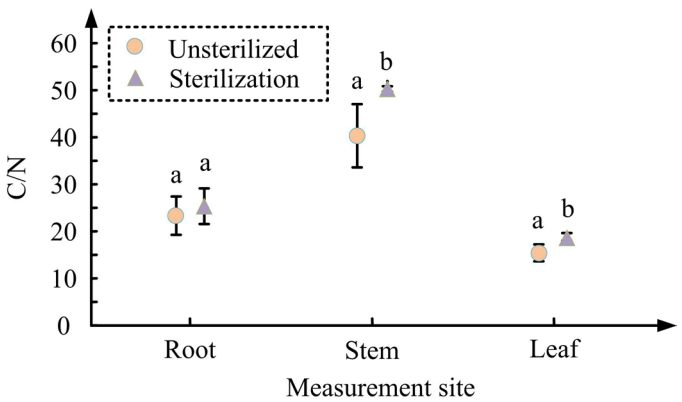

Figure 6. Inoculation with MF on C/N content.

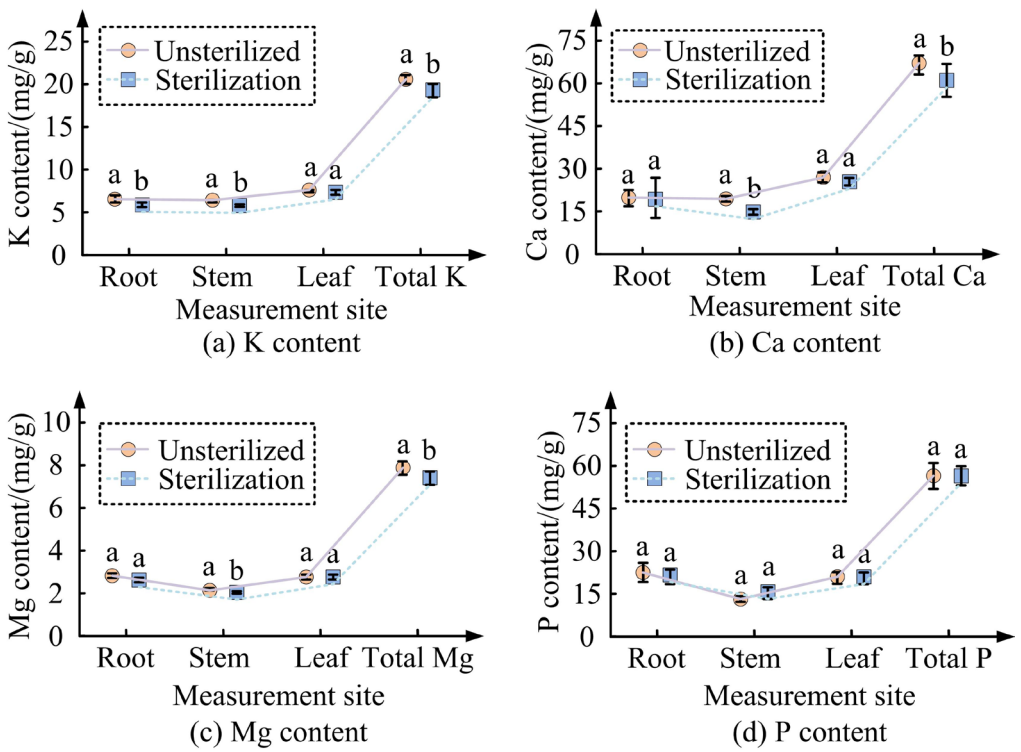

Figure 7. Effects of mycorrhizal inoculation on K, Ca, Mg, and P content.

leaves, roots, and stems is higher ($p < 0.05$). The content of leaf K is the highest, and MF has a very significant impact on leaf K content. The accumulation of Ca was high, with leaves accumulating the most Ca, 33% higher than the stem, and roots 32% higher, accounting for 40% of the total Ca. The accumulation of Ca in total Ca and stem, as well as the content of Ca in root-removed lines and leaves, was compared with the Cg ($p < 0.05$). The Ca content in the seedlings of *C. paliurus* was significantly different between different mycorrhizal treatments ($p < 0.05$). After *A. mycorrhizal* fungal treatment, the total Mg content of *C. paliurus* seedlings significantly decreased ($p < 0.05$). The accumulation of Mg in the roots of both groups of plants was greater than that in the leaves and stems, while the Mg in the stems was the least. The change in P content before and after mycorrhizal treatment was not significant, but the P content in the roots was 0.11 mg/g higher, and there was an increase. The accumulation of P in roots of each Tg was higher, i.e. 1.51 mg/g, about 40% of the total P. The accumulation of P in the control plant also underwent similar changes, but the accumulation of P in the stem was lower than that in the control plant, while the accumulation of P in the root was higher than that in the control plant. MF had no significant effect on the accumulation of plant P.

3.4. Effect of mycorrhizal inoculation on jasmonic acid content in *C. paliurus*

Figure 8 shows the analysis results of the influence of mycorrhizal inoculation on the content of jasmonic acid in *C. paliurus*.

From the variance analysis in Figure 8, it is seen that mycorrhizal inoculation has a significant impact on the content of jasmonic acid in *C. paliurus* leaves. The contents of jasmonic acid and jasmonic acid isoleucine in the nondisinfected group were higher. The content of jasmonic acid in the test group was 15.2 ng/g, while that in the Cg was 8.37 ng/g, twice as much as that in the former ($p < 0.05$). The concentration of jasmonic acid isoleucine in the two groups was 11.22 and 8.79 ng/g, 28% higher than that in the Cg ($p < 2\%$). Jasmonic acid is the sum of two kinds of jasmonic acid, 10.52 ng/g in the test group and 8.58 ng/g in the Cg, with 23% of the increase.

3.5. Effect of mycorrhizal inoculation on the content of SM in *C. paliurus*

Total flavonoids are an important natural metabolite that can not only exert disease resistance in plants but also have therapeutic effects in humans. The total triterpenes in plants can effectively enhance the body's natural immunity. Figure 9 shows the analysis of the details of inoculating mycorrhizal of total flavonoids and total triterpenes in *C. paliurus*.

As seen in Figure 9, the total flavonoid content in the roots of the nondisinfected Eg and the disinfected Cg was 4.48 and 4.35 mg/g, respectively. The stem of the former contained 3.17 mg/g, while the latter contained 2.42 mg/g. The former was 30% higher than the latter. The leaves of the former group contained 8.04 mg/g and those of the latter group 7.98 mg/g. The effect of mycorrhizal inoculation on the total triterpenoid content of *C. paliurus* seedlings was extremely obvious ($p < 0.05$). Caffeoylquinic acid is a polyphenolic acid widely present in nature that has various physiological activities, such as antiinflammatory and disease resistance. Figure 10 shows the quinic acid content of the *C. paliurus* seedlings.

According to Figure 10, the two Tgs did not have a significant impact on the content of caffeoylquinic acid in the seedlings of *C. paliurus*. The content of

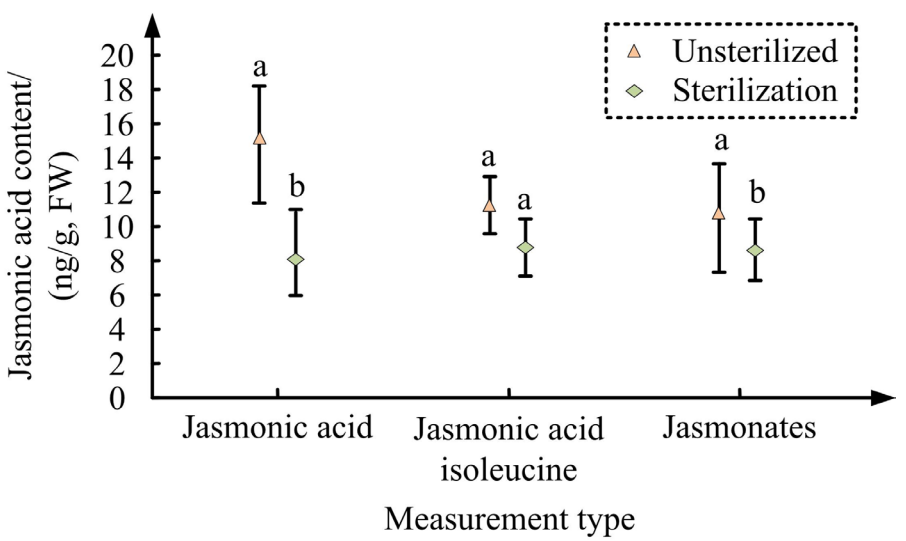

Figure 8. Effect of mycorrhizal inoculation on jasmonic acid content in *C. paliurus*.

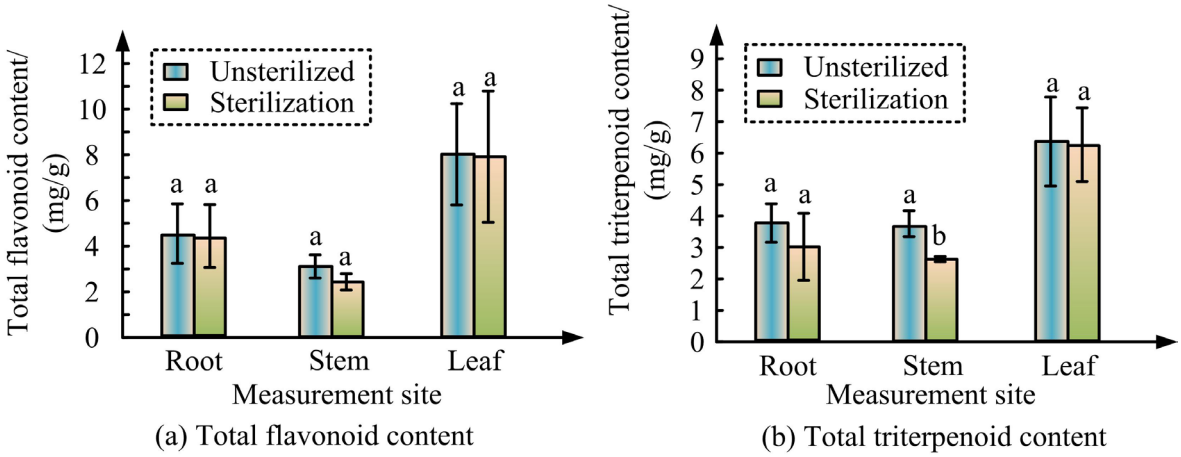

Figure 9. Effect of mycorrhizal inoculation on the content of total flavonoids and total triterpenoids in *C. paliurus*.

caffeoylquinic acid in the Eg and control rhizomes treated with nonsterilization was both 0.5 mg/g. The content of caffeoylquinic acid in the stems of the Eg and the Cg without disinfection was 0.116 mg/g and 0.131 mg/g, respectively, and the content of caffeoylquinic acid in the leaves was 0.209 and 0.142 mg/g, respectively. The Eg contained 47% more. Quercetin is a kind of flavonol compound and has a wide range of physiological activities. Kaempferol is a plant flavonoid monomer. The impact of mycorrhizal inoculation on the flavonoid monomer content of *C. paliurus* seedlings was analyzed and the results are listed in Figure 11.

As seen in Figure 11, mycorrhizal treatment had a prominent impact on the quercetin content of *C. paliurus* ($p < 0.05$), but the quercetin content in the root of the unsterilized test group was significantly lower ($p < 0.05$). The results showed that the content of quercetin was 0.012 mg/g in the Eg and 0.035 mg/g in the Cg, twice that in the Eg. Mycorrhizal treatment had no apparent effect on the content of kaempferol in the seedlings of *C. paliurus*. The content of kaempferol in the roots and leaves of the nondisinfected Eg was 0.079 and 0.043 mg/g, respectively, compared to the nondisinfected Cg, which was 85% higher than the Cg. This indicates that under different treatment conditions, the total flavonoid content between each treatment was 0.105 and 0.108 mg/g. The content of kaempferol in the leaves was 0.157 and 0.120 mg/g, respectively, both of which were 30% higher than those in the Cg.

3.6. Effect of mycorrhizal inoculation on the yield of SM in *C. paliurus*

Figure 12 shows the changes in quinic acid production of *C. paliurus* seedlings after inoculation with mycorrhizal fungi.

According to Figure 12, inoculation with mycorrhizal fungi has a very significant promoting effect on the yield of quinic acid in *C. paliurus* ($p < 0.05$). The quinic acid content in various parts of the green willow plants in the nondisinfected Eg was higher. The quinic acid content in the stems of the two groups was 0.467 mg/plant and 0.313 mg/plant, respectively. The Eg contained 49% more. Among them, the content of quinic acid in the leaves of cyclocarya paliurus is the highest, with a concentration of 0.868 mg/plant and 0.552 mg/plant. In the entire green willow of the Eg, the content of quinic acid was 1.525 mg, while in the Cg, it was 1.020 mg. In the Eg without disinfection, the yield of quinic acid in the stem was significantly different ($p < 0.05$). Afterward, the changes in flavonoid monomer yield of *C. paliurus* seedlings after inoculation with mycorrhizal fungi were analyzed, as expressed in Figure 13.

As seen in Figure 13, mycorrhizal treatment had a significant impact on the total flavonoid content of *C. paliurus* ($p < 0.05$); The content of total flavonoids in sterile *C. paliurus* was lower than that in the control, while in the stem and leaf it was higher, and the content of quercetin in the stem was higher ($p < 0.05$). The content of quercetin in both groups was 0.038 mg/plant and 0.095 mg/plant, which was significantly reduced by more than 50% compared with the control. In terms of the yield of stems and leaves, both the control and the Tg groups showed higher quercetin content in stems, reaching 0.298 mg/plant and 0.190 mg/plant, respectively, 56% higher. The content of quercetin in leaves reached 0.320 and 0.281 mg/plant respectively, slightly higher than that in the Cg. After mycorrhizal inoculation, the kaempferol in the roots of *C. paliurus* showed a large change: the content of quercetin in the roots, stems, and leaves of the test group and kaempferol in the stems were higher. Under the two treatments, the kaempferol in the root system was 0.181 and 0.116 mg/plant, which was 56% higher

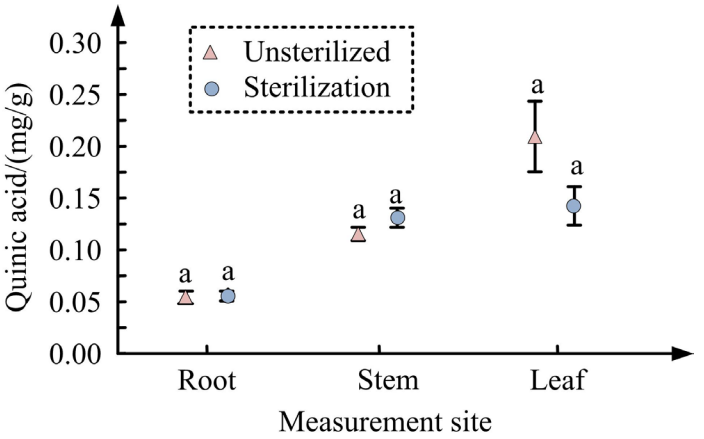

Figure 10. Effect of mycorrhizal inoculation on the content of caffeoylquinic acids in *C. paliurus*.

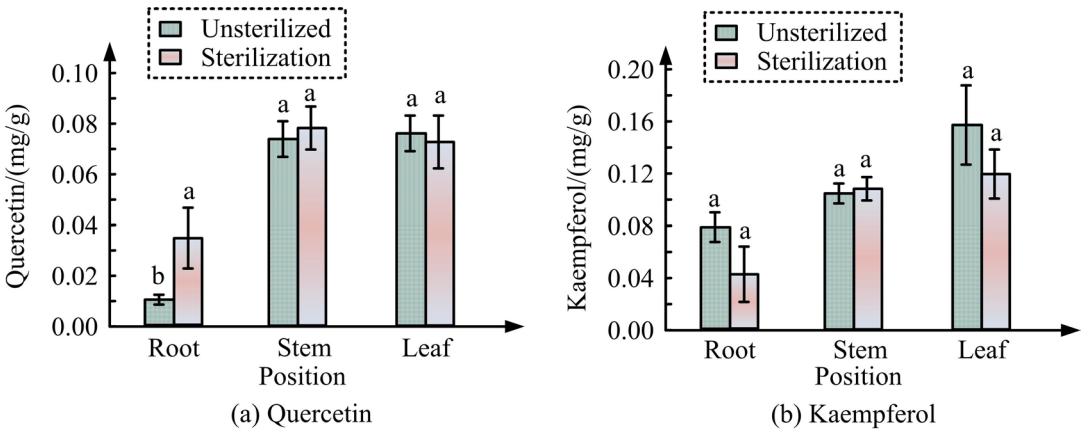

Figure 11. Effect of mycorrhizal inoculation on flavone monomer content in *C. paliurus* seedlings.

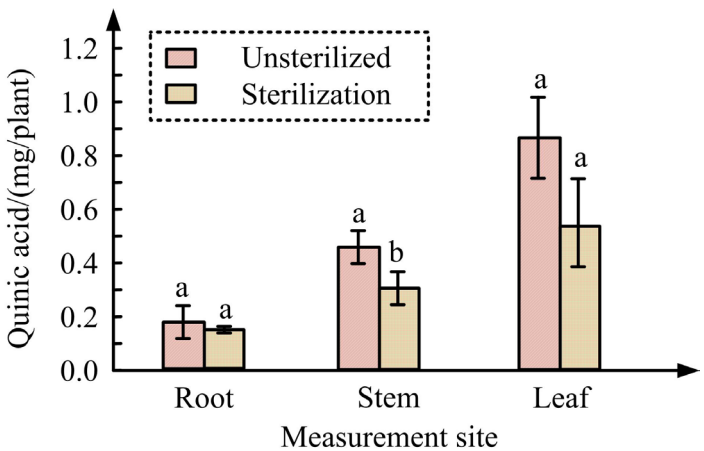

Figure 12. Changes in quinic acid production of *C. paliurus* seedlings after inoculation with mycorrhizal fungi.

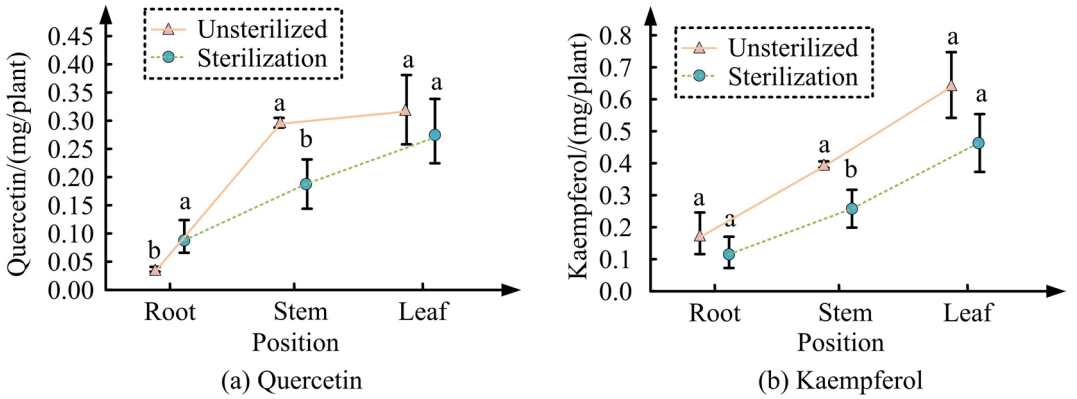

Figure 13. Changes in flavonoid monomer yield of *C. paliurus* seedlings after inoculation with mycorrhizal fungi.

than in the control; and 0.398 mg/plant and 0.260 mg/plant, respectively, in the stem, which is 53% higher than the control. Among them, the content of coumarin in the leaves is the highest, at 0.647 mg/plant and 0.463 mg/plant. The total yield of kaempferol from qing qian liu was 1.226 mg/plant and 0.839 mg/plant, respectively, which were 46% higher than in the control strain.

4. Conclusion

With the widespread application of *A. mycorrhizal* in forests, utilizing *A. mycorrhizal* to improve the survival rate of medicinal materials and ensure their quality has provided new ideas for the industrial production of *C. paliurus*. To reduce the amount of chemical fertilizer and balance the contradiction between the yield and quality of *C. paliurus* plantation, the aim of our study was to promote the growth of *C. paliurus* plantation and increase the production of its SM by inoculating AM. The experimental outcomes verified that MF inoculation significantly increased the total biomass of *C. paliurus* seedlings, and there was a significant distinction in biomass between *C. paliurus* seedlings inoculated with MF. In comparison with the Cg, the total biomass of the Eg was higher ($p < 0.05$), and the total biomass was 29.3% higher than that in the Cg. Inoculation with MF significantly promoted the yield of quinic acid and total flavonoid content in *C. paliurus* ($p < 0.05$). Subsequent research will analyze the correlation between mineral nutrients and SMs in *C. paliurus*.

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Author contributions

Wanxia Yang and Jiaqi Zhuang equally contributed to this work.

References

Abdullah L, Awang NA, Liow PT, Wan Mohd WR (2022). Optimal site for aquaculture farming: an elimination decision approach. Journal of Computational and Cognitive Engineering 2: 116-123. https://doi.org/10.47852/bonviewJCCE2202153

Ahmadi S (2022). Antibacterial and antifungal activities of medicinal plant species and endophytes. Cellular, Molecular and Biomedical Reports 2: 109-115. https://doi.org/10.55705/cmbr.2022.340532.1042

Almasi F (2021). Organic fertilizer effects on morphological and biochemical traits and yield in coriander (*Coriandrum sativum* L.) as an industrial and medicinal plant. Agrotechniques in Industrial Crops 1: 19-23. https://doi.org/10.22126/etic.2021.6476.1011

Arji I, Karimpour Kalehjoobi S, Nejatian MA, Upadhyay TK (2022). Yield and yield components of grapevines as influenced by mixed nano chelated fertilizer, humic acid and chemical fertilizers. Agrotechniques in Industrial Crops 2: 156-165. https://doi.org/10.22126/ATIC.2023.8478.1076https://doi.org/

Arji I, Safari M, Hadavi I (2021). Effects of different organic manures and chemical fertilizers on yield and yield component of olive (*Olea europaea* L.,) cv Zard in Kermanshah Province. Agrotechniques in Industrial Crops 1: 61-70. https://doi.org/10.22126/atic.2021.6514.1013

Aryafar S, Sirousmehr A, Najafi S (2021). The impact of compost on seed yield and essential oil of black cumin under drought stress conditions. Agrotechniques in Industrial Crops 1: 139-148. https://doi.org/10.22126/atic.2021.7184.1026

Chaudhary VB, Nolimal S, Sosa-Hernández MA, Ega C, Kastens J (2020). Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228: 238-252. https://doi.org/10.1111/nph.16667

Chen QL, Hu HW, Zhu D, Zhu YG, He JZ (2022). Calling for comprehensive explorations between soil invertebrates and arbuscular mycorrhizas. Trends in Plant Science 27: 793-801. https://doi.org/10.1016/j.tplants.2022.03.005

Crue T, Shipp J, Cohen SP, Leach J (2019). It's not easy being green: improvements to algae control in rice hydroponic growing systems. Phytopathology 109: S3.7. https://doi.org/10.1094/PHYTO-109-11-S3.1

Dou J, Paltakari J, Johansson LS, Vuorinen T (2019). Novel insight into the separation and composite utilization of sclerenchyma fiber bundles of willow bark. ACS Sustainable Chemistry & Engineering 7: 2964-2970. https://doi.org/10.1021/acssuschemeng.8b04001

Dueas JF, Camenzind T, Roy J, Hempel S, Rillig MC (2020). Moderate phosphorus additions consistently affect community composition of arbuscular mycorrhizal fungi in tropical montane forests in southern Ecuador. New Phytologist 227 (5): 1505-1518. https://doi.org/10.1111/nph.16641

Falaknaz M, Farokhian S, Kahrizi D (2022). Effect of foliar application of micronutrients on quantitative and qualitative characteristics of soybean (*Glycine max* L.). Agrotechniques in Industrial Crops 2: 79-86. https://doi.org/10.22126/atic.2022.7913.1059

Fu Y, Hou M, Zang H, Li H, Chin SL et al. (2019). Remote discrimination of willow, pine and poplar trees and their growing environments by femtosecond filament-induced breakdown spectroscopy. Spectrochimica Acta Part B Atomic Spectroscopy 155: 107-114. https://doi.org/10.1016/j.sab.2019.04.001

Jalilian S, Mondani F, Fatemi A, Bagheri A (2022). Evaluating the effect of farmyard manure and green manure on soil physicochemical traits and growth yield of organic sesame (*Sesamum indicium* L.). Agrotechniques in Industrial Crops 2: 19-31. https://doi.org/10.22126/atic.2022.7332.1039

Jean R, Khasa DP, Boudreau S (2021). Effects of one dark septate endophytic fungal and two Helotiales strains on the growth of plane-leaved willow (*Salix planifolia*) cuttings on iron ore waste rock. NRC Research Press 99: 725-733. https://doi.org/10.1139/cjb-2021-0043

Kakaeian AM, Mohammadi G. (2022). Azolla (Azollapinnata) response to different phosphorus and radiation treatments at two cultivation dates. Agrotechniques in Industrial Crops 2: 42-48. https://doi.org/10.22126/atic.2022.7364.1040

Khoshkhoo H, Rassam G, Babaeian M, Rahban S, Taghizadeh Tabari Z et al. (2022). The effect of foliar fertilizer and different growth regulators on quantitative and qualitative yields of saffron (*Crocus sativus* L.) in Farooj. Agrotechniques in Industrial Crops 2: 177-186. https://doi.org/10.22126/atic.2023.8410.1072

Kokkoris V, Lekberg Y, Antunes PM, Fahey C, Fordyce JA et al. (2020). Codependency between plant and arbuscular mycorrhizal fungal communities: what is the evidence? New Phytologist 228: 828-838. https://doi.org/10.1111/nph.16676

Nakamura M, Kumagai NH, Tamaoki M, Arita K, Yabe T (2020). Photosynthesis and growth of *Ulva ohnoi* and *Ulva pertusa* (Ulvophyceae) under high light and high temperature conditions, and implications for green tide in Japan. Phycological Research 68: 152-160. https://doi.org/10.1111/pre.12410

Norman JB, McManus M (2019). The techno-economic viability of bio-synthetic natural gas production utilising willow grown on contaminated land. AIMS Energy 7 (3): 285-312. https://doi.org/10.3934/energy.2019.3.285

Norouzi Y, Ghobadi M, Saeidi M, Dogan H. (2021). Effect of nitrogen and cytokinin on quantitative and qualitative yield of thyme (*Thymus vulgaris* L.). Agrotechniques in Industrial Crops 1: 52-60. https://doi.org/10.22126/etic.2021.6481.1012

Okiobe ST, Augustin J, Mansour I, Veresoglou SD (2019). Disentangling direct and indirect effects of mycorrhiza on nitrous oxide activity and denitrification. Soil Biology and Biochemistry 134: 142-151. https://doi.org/10.1016/j.soilbio.2019.03.025

Rahimian MH, Dadivar M, Zabihi HR (2021). The effect of three types of compost on grass water use efficiency. Agrotechniques in Industrial Crops 1: 197-203. https://doi.org/10.22126/atic.2022.7223.1029

Rillig MC, Aguilar-Trigueros CA, Anderson IC, Antonovics J, Bergmann J et al. (2020). Myristate and the ecology of AM fungi: significance, opportunities, applications and challenges. New Phytologist 227: 1610-1614. https://doi.org/10.1111/nph.16527

Rondina A, Tonon BC, Lescano L, Hungria M, Lmogueira MA et al. (2019). Plants of distinct successional stages have different strategies for nutrient acquisition in an Atlantic rain forest ecosystem. International Journal of Plant Sciences 180: 186-199. https://doi.org/10.1086/701353

Rostami Ahmadvandi H, Faghihi A (2021). Adapted oilseed crops with the ability to grow economically in dryland conditions in Iran. Agrotechniques in Industrial Crops 1: 122-128. https://doi.org/10.22126/atic.2021.6518.1015

Salehi-Sardoei A, Babarabi M, Ghasemi H (2023). Vermicompost as growing media replacement for *Polianthes tuberosavar* pearl production. Agrotechniques in Industrial Crops 3: 44-52. https://doi.org/10.22126/atic.2023.9121.1096

Salehi-Sardoei A, Khalili H (2022). Nitric oxide signaling pathway in medicinal plants. Cellular, Molecular and Biomedical Reports 2: 1-10. https://doi.org/10.55705/cmbr.2022.330292.1019

Standish RJ, Albornoz FE, Morald TK, Hobbs RJ, Tibbett M (2021). Mycorrhizal symbiosis and phosphorus supply determine interactions among plants with contrasting nutrient-acquisition strategies. Journal of Ecology 109: 3892-3902. https://doi.org/10.1111/1365-2745.13766

Stolarski M, Szczukowski S, Tworkowski J, Krzyaniak M (2019). Extensive willow biomass production on marginal land. Polish Journal of Environmental Studies 28: 4359-4367. https://doi.org/10.15244/pjoes/94812

Tabin S, Gupta RC, Kamili AN, Parray JA (2022). Medical and medicinal importance of *Rheum* spp. collected from different altitudes of the Kashmir Himalayan range. Cellular, Molecularand Biomedical Reports 2: 187-201. https://doi.org/10.55705/cmbr.2022.349901.1050

Veresoglou SD, Chen B, Fischer MM, Helgason T, Mamolos AP et al. (2019). Latitudinal constraints in responsiveness of plants to arbuscular mycorrhizal: the 'sun-worshipper' hypothesis. New Phytologist 224: 552-556. https://doi.org/10.1111/nph.15918

Wang P, Jiang H, Boeren S, Dings H, Kulikova O et al. (2021). A nuclear-targeted effector of *Rhizophagus irregularis* interferes with histone 2B mono-ubiquitination to promote arbuscular mycorrhization. New Phytologist 230: 1142-1155. https://doi.org/10.1111/nph.17236

Wang X, Fang L, Beiyuan J, Cui Y, Zhang X (2021). Improvement of alfalfa resistance against Cd stress through rhizobia and arbuscular mycorrhiza fungi co-inoculation in Cd-contaminated soil. Environmental Pollution 277: 116758.1-116758.12. https://doi.org/10.1016/j.envpol.2021.116758

Wipf D, Krajinski F, Tuinen D, Recorbet G, Courty P (2019). Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. New Phytologist 223 (3): 1127-1142. https://doi.org/10.1111/nph.15775

Zhurbenko MP, Ohmura Y (2020). Contributions to the knowledge of lichenicolous fungi growing on baeomycetoid lichens and *Icmadophila*, with a key to the species. The Lichenologist 52 (6): 437-453. https://doi.org/10.1017/S002428292000047X