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# Mediating Effect of Bio-Organic Fertilizer on the Physiological Characteristics of "Qi-Nan" Agarwood from *Aquilaria sinensis* (Lour.)

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**Abstract:** This study examines the impact of bio-organic fertilizers on the physiological characteristics and leaf nutrient content of "Qi-Nan" agarwood seedlings. The aim is to provide a theoretical foundation for the efficient cultivation of "Qi-Nan". The experiment was conducted under field conditions using a Randomized complete block design. The control group received no fertilization, while the three fertilizer-treated groups received vermicompost, sheep manure, or microbial organic fertilizer. The results indicate that the application of bio-organic fertilizer significantly promoted the growth of plant height and diameter, increased the contents of soluble proteins, soluble sugar, chlorophyll, and leaf nutrients; and decreased the contents of free proline, peroxidase, malondialdehyde, and other stress indices. Redundancy analysis showed that the main factors affecting the seedlings height and diameter were free proline, chlorophyll, total nitrogen, and total potassium. Principal component analysis and membership function analysis revealed significant variations in growth, physiological characteristics, and nutrient content among the three fertilizer groups and the control group, with vermicompost > microbial organic fertilizer > sheep manure > no fertilization. In conclusion, biological organic fertilizer significantly promotes the growth of "Qi-Nan" seedlings, and vermicompost is the most effective fertilizer in this experiment.

**Keywords:** agarwood; Qi-Nan; *Aquilaria sinensis*; bio-organic fertilizer; physiological and biochemical indices; nutrient element content



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## 1. Introduction

Agarwood (Chen-Xiang in Chinese) is a valuable resinous heartwood from the *Aquilaria* species of the Thymelaeceae family [1]. It is widely used in medicine, aromatherapy, the perfume industry, religious culture, and other fields. It is considered a traditional medicine and natural spice [2–4]. "Qi-Nan" is also known as Kanankoh, Kyara [5,6], or Chi Nan [7]. The wild "Qi-Nan" is usually considered to be the highest quality wild agarwood, and its price is thousands of times higher than ordinary agarwood [8]. However, due to the destruction of the natural environment and excessive deforestation caused by economic interests, wild *Aquilaria* species are endangered [9]. This genus is now listed in Appendix II of the Convention on Internal Trade in Endangered Species of Wild Fauna and Flora [10]. Additionally, the low yield of wild *Aquilaria* species cannot meet the market demand. As a result, planting agarwood trees has become an important way to obtain agarwood. In recent years, grafted "Qi-Nan" agarwood has become available, which is a high-quality agarwood obtained by grafting and breeding from natural wild agarwood trees [11,12]. *Aquilaria sinensis* "Reke2"is an example of high-quality cultivated "Qi-Nan" propagated by grafting with the ordinary germplasm of *Aquilaria sinensis* [13]. It has been cultivated in places such as Hainan, Guangdong, Guangxi, and Yunnan, with excellent results. Compared to trees grown from ordinary agarwood seedlings, grafted "Qi-Nan" agarwood can more easily produce higher-quality agarwood and has the advantages of early incense-bearing and high yield [11,14]. In order to meet the demand of the agarwood market, China is vigorously promoting the planting of "Qi-Nan". According to preliminary statistics, 5766 acres of "Qi-Nan" seedlings have been planted in the country [15–17]. Fertilization is a key measure for seedling planting [18] and is essential for the healthy growth of trees, which is the basis for forming more agarwood.

The misuse of chemical fertilizer is causing significant negative effects globally, such as air pollution, eutrophication, soil acidification, and various ecological and environmental problems [19–21]. In recent years, organic fertilizers have been used as a replacement for chemical fertilizers in various proportions as an important measure to curb the overuse of chemical fertilizers and accelerate the development of sustainable agriculture. Bio-organic fertilizer is a type of fertilizer that combines the benefits of microbial and organic fertilizers [22]. It is composed of specific functional microorganisms and organic materials primarily derived from plant and animal residues [22,23]. The application of bio-organic fertilizers can increase soil organic matter [24], increase fertility factors such as soil fertility and water retention, improve soil microbiota, increase enzyme activity in the soil, and enhance the comprehensive defense capability of plants against adverse environments [25,26]. Compared with traditional organic fertilizers, bio-organic fertilizers are more effective in promoting tree growth and development [27]. It has been widely used in precious tree species. For example, bio-organic fertilizer had a greater effect on the rejuvenation of Bayberries (*Myrica rubra*) with decline disease [28]; bio-organic fertilizer can also increase the number of microorganisms and the microecological index in *Pinus koraiensis* [29].

In terms of fertilization for *Aquilaria* species, some researchers have studied the fertilization methods of *A. sinensis*. For example, a combination of 10 g bio-fertilizer and 20 g chemical fertilizer was found to be the optimal formula to promote the growth of *A. sinensis* and improve soil fertility [30]; the application of a mixture of inorganic fertilizer, organic fertilizer, and bio-fertilizer was recommended to promote the growth of *A. sinensis* seedlings [31]. In addition, the fertilization method of *A. malaccensis* has also been preliminarily explored. At a nitrogen fertilization level of 3000 mg/seedlings, the photosynthetic physiology characters and biomass of *Aquilarias* seedlings reached their maximums [32]; the optimal combination was found to be 3.637 $g \cdot plant^{-1}$ of N fertilization + 2.50 $g \cdot plant^{-1}$ of P fertilization + 1.25 $g \cdot plant^{-1}$ of K fertilization, under which the biomass of *A. malaccensis* seedlings reached 27.688 g [33]. However, there is currently a lack of research on the fertilization and growth of "Qi-Nan" seedlings. For example, the effects of different fertilization methods on the physiology and nutrient content of grafted "Qi-Nan" agarwood seedlings have not been reported. Given that the quality and yield of "Qi-Nan" agarwood are far from meeting the market demand, finding an efficient and reasonable fertilization method for breeding seedlings is a crucial problem in the current cultivation process of "Qi-Nan" agarwood seedlings that urgently needs to be addressed.

Thus, this study aimed to investigate the effects of environment-friendly fertilizers such as vermicompost, sheep manure, and microbial organic fertilizers on the physiological characteristics and nutrient content of "Qi-Nan" agarwood seedlings. The objectives of this study were as follows: (1) to examine the changes in the physiology and nutrient content of "Qi-Nan" seedlings under different bio-organic fertilizer treatments; (2) to identify which physiological and nutrient indices were the key factors influencing the height and stem diameter of "Qi-Nan" seedlings; and (3) to propose a suitable bio-organic fertilizer for "Qi-Nan" seedlings.

## 2. Materials and Methods

### *2.1. Test Materials*

In May of 2021, a field test was conducted by transplanting young "Qi-nan" trees from the Nalou Township nursery base in Nanning City, Guangxi Zhuang Autonomous Region (located at 108°63′ E, 22°60′ N) to the Shaoping Forest Farm Centre in Xia Shi Township, Pingxiang City, Guangxi Zhuang Autonomous Region (located at 106°90 E, 22°12′ N). The young trees were selected based on their consistent growth, with an average diameter of 0.30 ± 0.08 cm and an average height of 0.42 ± 0.12 m. The area where the trial was conducted belongs to the subtropical monsoon climate zone in southern Asia and has an annual average rainfall of 1200–1500 mm and an average temperature of 20.5–21.7 °C [34].

The test fertilizers were vermicompost (VC), sheep manure (SM), and microbial organic fertilizer (MOF), with no fertilizer (CK) as the control. VC is known as the king of organic fertilizer, and its large amounts of microorganisms can effectively inhibit the reproduction of harmful pathogens [35]. SM is more effective in improving soil, and it contains more organic matter than other animal dung and has stronger fertility [36], so it is more suitable to be used as a base fertilizer. MOF is a water-soluble fertilizer containing amino acids, which is more widely used as a functional water-soluble fertilizer in the cultivation of large fields [37]. These three fertilizers are all representative. Furthermore, VC, SM and MOF have rarely been studied in plants of the Thymelaeceae family, and even less researched has analyzed these three fertilizers in comparison with each other. Therefore, VC, SM, and MOF were selected as the base fertilizers in this experiment. Among them, VC was produced by Guangxi yanhe Agricultural Technology Co., LTD, with a relative humidity of 65%–75% and a temperature of 15–25 °C; the Eisenia foetida used were raised with semi-decomposed cow manure. After six months of cultivation, the grey matter left behind by the earthworm was used as the earthworm manure in this experiment. The SM was produced by the Ruilin Center of the Chinese Academy of Forestry, and the MOF was developed by the Juju Research Institute of the Chinese Academy of Agricultural Sciences. MOF is an amino acid fertilizer, with amino acid ≥ 10%, humic acid and trace element ≥ 2.5%, and effective viable count ≥ 2 × $10^8$ CFU/g. The basic properties of the three fertilizers are shown in Table 1. The soil used was Latosolic Red Soil and is classified as Hapludults according to the USDA soil taxonomy [38,39]. The essential physical and chemical properties are shown in Table 2. Latosolic Red Soil is acidic soil suitable for the growth of agarwood. Nitrogen (pure N) was applied at 600 mg/plant in each treatment [40]. Nitrogen fertilizer was applied in equal amounts per plant according to the nitrogen content of the fertilizer. The amount of fertilizer applied is converted according to the nutrient content measured in Table 1.

**Table 1.** Experimental fertilizer treatments properties.

| Fertilizer | Total N | Total P | Total K | Total Amount of Nutrients | Organic Matter | Moisture Content | pH Value |
|---|---|---|---|---|---|---|---|
| | % | | | | | | |
| CK | – | – | – | – | – | – | – |
| VC | 2.11 | 1.57 | 1.84 | 5.52 | ≥45 | 51.79 | 6.70 |
| SM | 1.53 | 1.12 | 1.09 | 3.74 | ≥45 | 60.06 | 8.40 |
| MOF | 1.62 | 1.86 | 1.95 | 5.43 | ≥45 | 53.54 | 5.50 |

No fertilizer (CK); Vermicompost (VC); Sheep manure (SM); Microbial organic fertilizer (MOF).

**Table 2.** Some important physicochemical properties of soil used in the study.

| Parameters | Organic Matter | Total N | Total P | Total K | pH Value |
|---|---|---|---|---|---|
| | $g{\cdot}kg^{-1}$ | | | | |
| values | 16.13 | 1.02 | 0.30 | 1.15 | 4.97 |

*2.2. Experimental Design*

The experiment was conducted in a randomized complete block design, with a row spacing of 1.5 m × 1.5 m. There were four treatments: VC, SM, MOF, and CK. A strip fertilization ditch of 30 cm in depth, 20 cm in width, and 15 cm in length was dug at a distance of 5–6 cm from the plant for fertilization. The bio-organic fertilizer was mixed with the soil in the trench and applied, then the trench was backfilled with 1–2 cm of topsoil. The bio-organic fertilizer was applied as a base fertilizer all at once. Other field management practices in the trial field were kept consistent. Prior to fertilizing "Qi-nan," the bags of three different types of fertilizer remained open and were left in the same room or workshop for a certain period of time. This ensures that all three types of fertilizer have the same level of humidity and temperature before being applied to the plants. Fertilizer was applied once in May 2021, and the experiment ended in May 2022 for 12 months. Each treatment plot was 600 $m^2$ in size, and three 10 m $\times$ 10 m sample squares were drawn in the middle of the plot. All 45 seedlings planted in each sample square were marked, i.e., 135 plants were marked within a treatment plot. All labelled seedlings were monitored every 3 months to measure their plant height and stem diameter, and from these seedlings, well-grown ones were selected and leaves were collected to measure their physiological and nutritional indicators.

### *2.3. Sample Collection and Determination*

#### 2.3.1. Determination of Growth Index

The plant height was measured with a straightedge from the plant stem diameter to its terminal bud, accurate to 0.1 cm. The stem diameter was measured at the graft union, accurate to 0.1 cm. Measurements were made five times in May 2021, August 2021, November 2021, February 2022, and May 2022, each at the exact location.

#### 2.3.2. Determination of Physiological Indices and Leaf Nutrient Content

For each treatment, three well-grown seedlings were randomly selected, and the upper branches were used to count the 2nd to 5th mature functional leaves from the terminal bud downwards. These leaves were wiped clean, put into plastic bags, labeled, and temporarily stored in ice boxes; after collection, they were brought back to the laboratory and stored in an ultra-low-temperature refrigerator ($-70$ °C). The contents of chlorophyll, soluble sugar, soluble protein, free proline, and malondialdehyde were determined by the acetone extraction method [41], anthrone colorimetric method [42], Coomassie brilliant blue method [43], acid ninhydrin colorimetric method [44], and thiobarbituric acid colorimetric method [42], respectively. The activities of superoxide dismutase and peroxidase were determined by acid ninhydrin colorimetry [45] and guaiacol colorimetry [42]. The instruments used for the above indicators were all enzyme-labeled instruments. The mature functional leaves were rinsed with distilled water, blotted dry with absorbent paper, placed in an envelope bag, and oven-dried at 105 °C for half an hour, followed by continuous baking at 75 °C for 48 h until constant weight. After grinding, the leaves were passed through a 0.15 mm sieve, and 0.1 g of dry powder was taken per replicate. After $H_2SO_4$-$H_2O_2$ decoction, total nitrogen, total phosphorus, and total potassium were determined by Kjeldahl, molybdenum antimony anti-colorimetric, and flame photometric methods, respectively [46]. The instruments used were nitrogen digestion, an enzyme-labeled instrument, and a flame photometer. The measurements were performed three times in November 2021, February 2022, and May 2022.

### *2.4. Data Analysis and Processing Methods*

SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses by ANOVA and significance tests. $p \leq 0.05$ was considered to indicate statistically significant differences. The purpose of PCA in CANOCO version 4.5 (Ithaca, NY, USA) was used to examine the effects of fertilizer on the physiological characteristics of "Qi-Nan" agarwood. Redundancy analysis (RDA) was used to determine the relationship between the fertilization treatment group and the measured index using CANOCO 4.5 software (Ithaca, NY, USA). Mantel tests were employed to compute the correlation between the growth and physiological characteristics of "Qi-Nan" agarwood. Then, the fuzzy membership function method is used for comprehensive evaluation.

## 3. Results

### 3.1. Effect of Different Bio-Organic Fertilizer Treatments on the Growth of "Qi-Nan" Agarwood Seedlings

Bio-organic fertilization significantly increased the growth of the seedlings with respect to the control (Figure 1). The height and stem diameter of "Qi-Nan" agarwood seedlings were significantly different between groups ($p \leq 0.05$). Under different fertilization treatment groups, the dynamic changes in plant height and stem diameter growth of "Qi-Nan" agarwood seedlings were the same and showed an upward trend. The growth rhythm was characterized by "slow-fast-slow" and showed an S-shaped curve. The overall order is VC > SM > MOF > CK. The height and stem diameter both obtained maximum values at VC, reaching 1.38 and 1.45 times that of CK, respectively. In conclusion, VC was the best fertilizer to promote the growth of plant height and stem diameter of "Qi-Nan" agarwood seedlings.

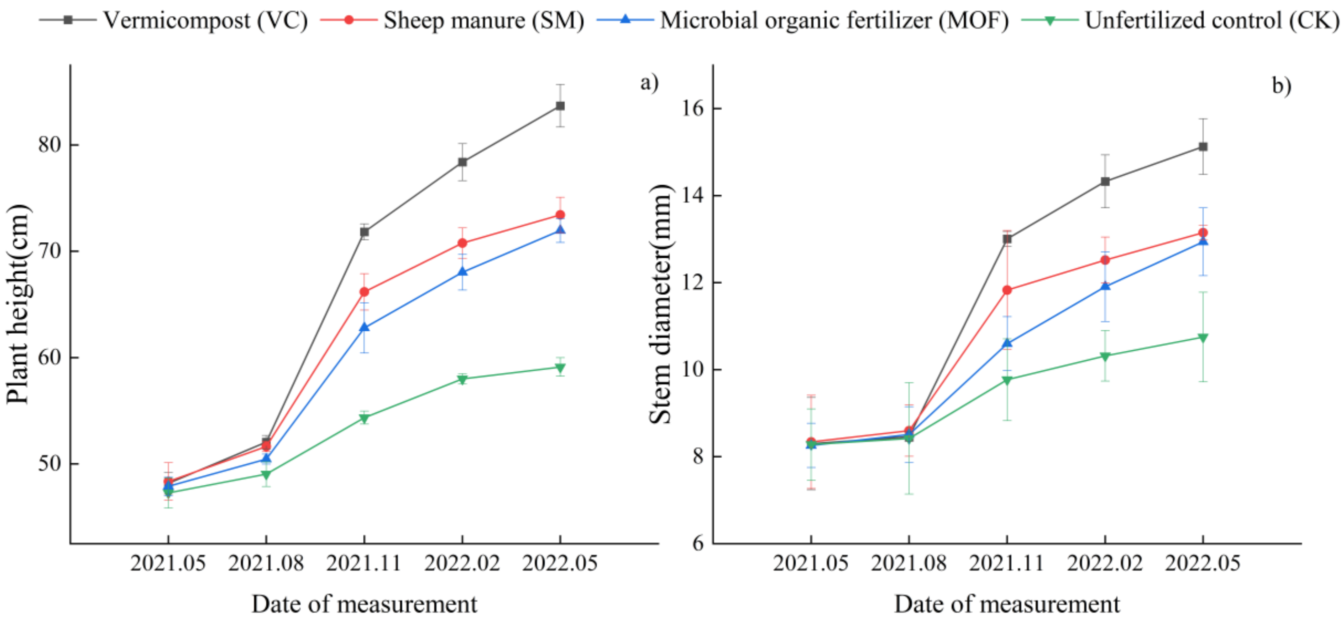


**Figure 1.** Growth rhythms of height and stem diameter of "Qi-Nan" agarwood seedlings under different bio-organic fertilizer treatments. (**a**) plant height; (**b**) stem diameter. No fertilizer (CK); vermicompost (VC); sheep manure (SM); microbial organic fertilizer (MOF). The bars are means ± standard error.

### 3.2. Effect of Different Bio-Organic Fertilizer Treatments on Physiological and Biochemical Indices of "Qi-Nan" Agarwood Seedlings

#### 3.2.1. Effect of Bio-Organic Fertilizer Treatments on the Content of Osmotic Mediating Substances in "Qi-Nan" Agarwood Seedlings

Figure 2a shows the effects of four fertilization treatments on soluble protein (SP) in "Qi-Nan" agarwood seedlings. In February 2021 and May 2022, the differences among all treatment groups were significant ($p \leq 0.05$) and showed a trend of first increasing and then decreasing with time. In November, the SP content in the VC group was the best, which increased by 22.63% compared with the CK group. In February 2022 and May 2022, the SM group was the best, which increased by 56.00% and 50.00% compared with the CK group.

Figure 2b shows the effects of four fertilization treatments on soluble sugar (SS) in "Qi-Nan" agarwood seedlings. In November 2021, February 2022, and May 2022, there were significant differences among the SS groups of "Qi-Nan" agarwood seedlings ($p \leq 0.05$), and all groups showed a decreasing trend with time. In November 2021, the VC group was the best, as it increased by 48.35% compared to the CK group. In February 2022 and May 2022, the MOF group was the best, as it increased by 47.50% and 56.00% compared with the CK group.

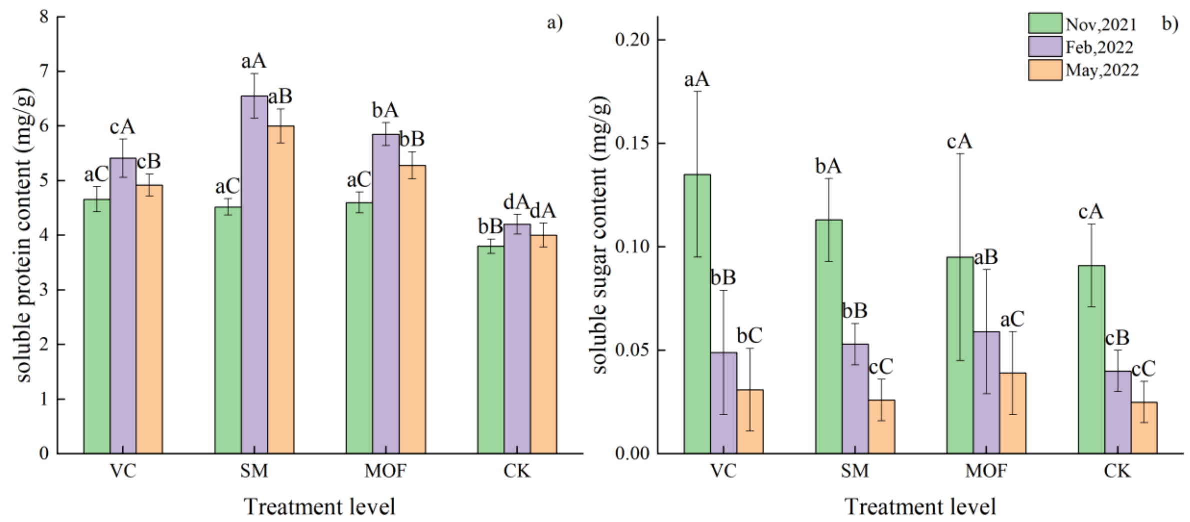


**Figure 2.** Effects of different bio-organic fertilizer treatments on soluble protein and soluble sugar contents of "Qi-Nan" agarwood seedlings. (**a**) soluble protein content; (**b**) soluble sugar content. No fertilizer (CK); vermicompost (VC); sheep manure (SM); microbial organic fertilizer (MOF). The bars were means ± standard error. Capital letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same treatment and different periods, and lowercase letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same period and different treatments.

3.2.2. Effect of Bio-Organic Fertilizer Treatments on the Content of Free Proline and Malondialdehyde in "Qi-Nan" Agarwood Seedlings

Bio-organic fertilized "Qi-Nan" trees had lower malondialdehyde (MDA) content than the control (Figure 3a). In November 2021, February 2022, and May 2022, there was a difference in the MDA content of "Qi-Nan" agarwood seedlings among groups ($p \leq 0.05$), and all groups showed a trend of increasing first and then decreasing with time. In February and May, the VC group was the smallest, at 2.83 µmol/gFW and 2.26 µmol/gFW less than the CK group.

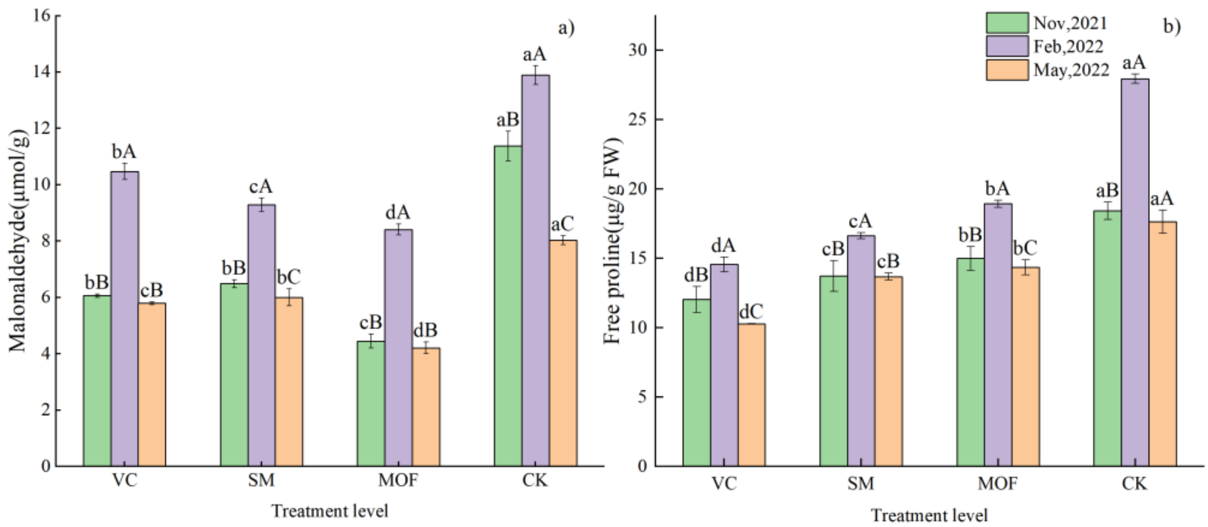


**Figure 3.** Effects of different bio-organic fertilizer treatments on Malonaldehyde and Free proline contents of "Qi-Nan" agarwood seedlings. (**a**) malonaldehyde; (**b**) free proline. No fertilizer (CK); vermicompost (VC); sheep manure (SM); microbial organic fertilizer (MOF). The bars were means ± standard error. Capital letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same treatment and different periods, and lowercase letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same period and different treatments.

Bio-organic fertilized "Qi-Nan" trees had lower free proline (FP) content than the control (Figure 3b). In November 2021, February 2022, and May 2022, there was a difference in the MDA content of "Qi-Nan" agarwood seedlings among groups ($p \leq 0.05$), and all groups showed a trend of first increasing and then decreasing with time. The average VC group was the smallest, at 6.37 µg/gFW, 13.38 µg/gFW, and 7.35 µg/gFW less than the CK group.

### 3.2.3. Effect of Bio-Organic Fertilizer Treatments on Enzyme Activity of "Qi-Nan" Agarwood Seedlings

In November 2021, February 2022, and May 2022, there were differences in the superoxide dismutase activity (SOD) content among groups ($p \leq 0.05$) (Figure 4a), and all groups showed an increasing trend with time. In November 2021, the SM group was the best, increasing by 73.66% compared with CK. In February 2022 and May 2022, the MOF group was the largest, rising by 64.01% and 60.78% compared with the CK group.

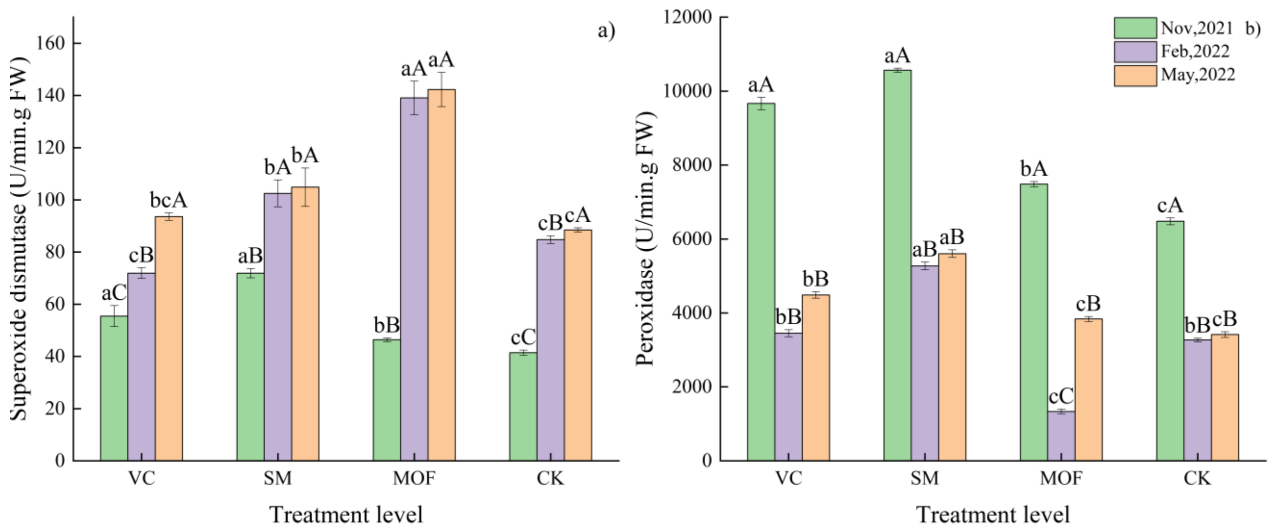


**Figure 4.** Effects of different bio-organic fertilizer treatments on superoxide and peroxidase activities of "Qi-Nan" agarwood seedlings. (**a**) superoxide dismutase; (**b**) peroxidase. No fertilizer (CK); vermicompost (VC); sheep manure (SM); microbial organic fertilizer (MOF). The bars were means ± standard error. Capital letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same treatment and different periods, and lowercase letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same period and different treatments.

In November 2021, February 2022, and May 2022, the peroxidase activity (POD) content of "Qi-Nan" agarwood seedlings was different among groups ($p \leq 0.05$) (Figure 4b). Each group showed a trend of decreasing and then slightly increasing over time. The SM group was the best and was 163.00%, 162.90%, and 164.14% higher than the CK group.

### 3.2.4. Effect of Bio-Organic Fertilizer Treatments on Chlorophyll Content of "Qi-Nan" Agarwood Seedlings

The application of bio-organic fertilizer significantly increased the content of chlorophyll in "Qi-Nan" agarwood seedlings compared with CK (Figure 5). There was a difference in the chlorophyll content of "Qi-Nan" agarwood seedlings among groups ($p \leq 0.05$). All groups showed a rising trend over time. In November 2021, February 2022, and May 2022, the VC group was the best, increasing by 158.54%, 134.06%, and 137.73% compared with the CK group.

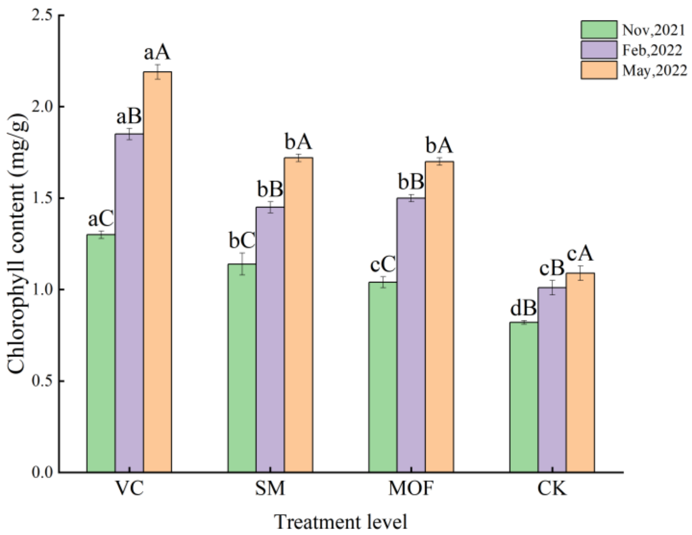


**Figure 5.** Effects of different bio-organic fertilizer treatments on chlorophyll content of "Qi-Nan" agarwood seedlings. No fertilizer (CK); vermicompost (VC); sheep manure (SM); microbial organic fertilizer (MOF). Capital letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same treatment and different periods, and lowercase letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same period and different treatments.

### *3.3. Effect of Bio-Organic Fertilizer Treatments on the Nutrient Content of "Qi-Nan" Agarwood Seedlings*

According to Table 3, under different fertilization methods and different seasons, the total nitrogen (TN), total phosphorus (TP), and total potassium (TK) of leaves of "Qi-Nan" agarwood seedlings were different ($p \leq 0.05$), and all fertilization treatments showed higher amounts of these minerals than CK. Among them, TN and TK showed a rising trend with the fertilization process, and TP showed a decreasing and then increasing trend with the fertilization process. After the fertilization experiment, the contents of TN, TP, and TK in leaves were the highest in the VC group, which were increased by 10.33 g/kg, 0.021 g/kg, and 8.04 g/kg compared with the CK group, respectively.

**Table 3.** Effects of different bio-organic fertilizer treatments on the nutrient content of "Qi-Nan" agarwood seedlings.

| The Nutrient Contents | Treatment | December 2021 | February 2022 | May 2022 |
|---|---|---|---|---|
| Total nitrogen content in leaves (TN, g/kg) | VC | 17.69 ± 0.93 aB | 19.86 ± 1.69 aB | 23.63 ± 1.25 aA |
| | SM | 12.27 ± 2.23 bcB | 14.36 ± 0.88 abB | 18.83 ± 1.69 bA |
| | MOF | 14.43 ± 0.61 abA | 14.52 ± 1.70 abA | 17.27 ± 0.69 bA |
| | CK | 9.20 ± 0.28 cB | 10.55 ± 0.99 bB | 13.30 ± 0.64 cA |
| Total phosphorus content in leaves (TP, g/kg) | VC | 0.047 ± 0.02 aA | 0.019 ± 0.01 bC | 0.040 ± 0.01 aB |
| | SM | 0.048 ± 0.01 aA | 0.021 ± 0.01 bC | 0.034 ± 0.01 bB |
| | MOF | 0.041 ± 0.01 aA | 0.023 ± 0.01 aB | 0.036 ± 0.01 bB |
| | CK | 0.029 ± 0.01 bA | 0.012 ± 0.01 cC | 0.019 ± 0.01 cB |
| Total potassium content in leaves (TK, g/kg) | VC | 7.85 ± 0.15 aC | 9.06 ± 0.66 aB | 14.91 ± 0.26 aA |
| | SM | 6.45 ± 0.13 bB | 7.20 ± 0.20 bAB | 8.14 ± 0.90 cA |
| | MOF | 5.75 ± 0.69 bC | 7.93 ± 0.98 bB | 10.84 ± 0.64 bA |
| | CK | 5.81 ± 0.06 bB | 6.15 ± 1.17 cB | 6.87 ± 0.48 dA |

Capital letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same treatment and different periods, and lowercase letters represent significant differences at the 0.05 level ($p \leq 0.05$) for the same period and different treatments. Values are means ± standard errors (n = 9). No fertilizer (CK); Vermicompost (VC); Sheep manure (SM); Microbial organic fertilizer (MOF).

## 4. Comprehensive Analysis of All the Measured Indices of "Qi-Nan" Agarwood Seedlings

Principal component analysis (PCA) was used to analyze 12 physiological and nutrient content indices of "Qi-Nan" agarwood seedlings grown using different bio-organic fertilizers (Figure 6). The cumulative variance contribution rate of PC1 and PC2 was 79.9%, and most of the information of the 12 variables could be reflected by them. The samples at the early growth stage (November 2021) were distributed in the lower right corner, while those at the late growth stage (February 2022 and May 2022) were distributed in the upper right corner and upper left corner, indicating that there were significant differences in physiology and nutrient content between the seedlings at the early and late growth stages. In addition, no matter the stage of development, the distance between the non-fertilized group (CK) and the fertilized group was more significant. They are distributed in different areas without overlap, indicating that the physiological and nutrient indices of "Qi-Nan" agarwood seedlings are pretty different under fertilization and no fertilization. Applying fertilizer significantly affects "Qi-Nan" agarwood seedlings' growth and development.

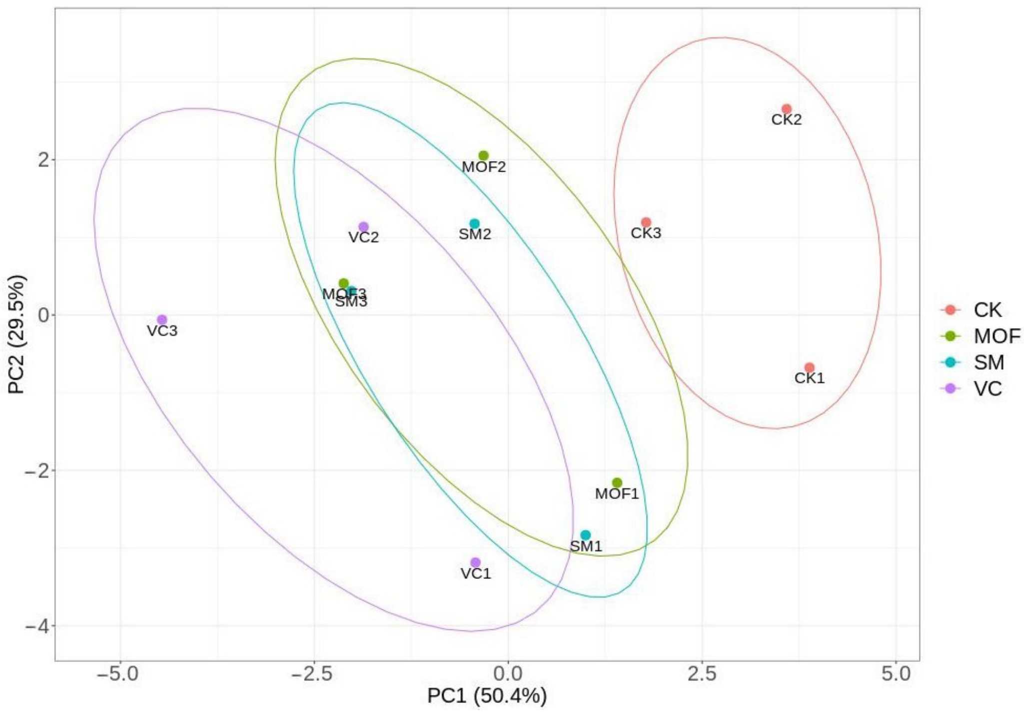


**Figure 6.** Principal component analysis of "Qi-Nan" agarwood seedlings under different fertilizer treatments: No fertilizer (CK); vermicompost (VC); sheep manure (SM); microbial organic fertilizer (MOF). The number represents the measured value in different periods, where 1 represents the measured value in November 2021, 2 represents the measured value in February 2022, and 3 represents the measured value in May 2022.

In a redundancy analysis of the fertilizer treatment groups and the 12 indicators for "Qi-Nan" agarwood seedlings, the first axis could explain 79.46% of all information, the second axis could explain 0.22%, and the cumulative amount of information was 79.68% (Figure 7). It can be seen that the relationship of each indicator is reflected by the first two axes well and is mainly determined by the first axis.

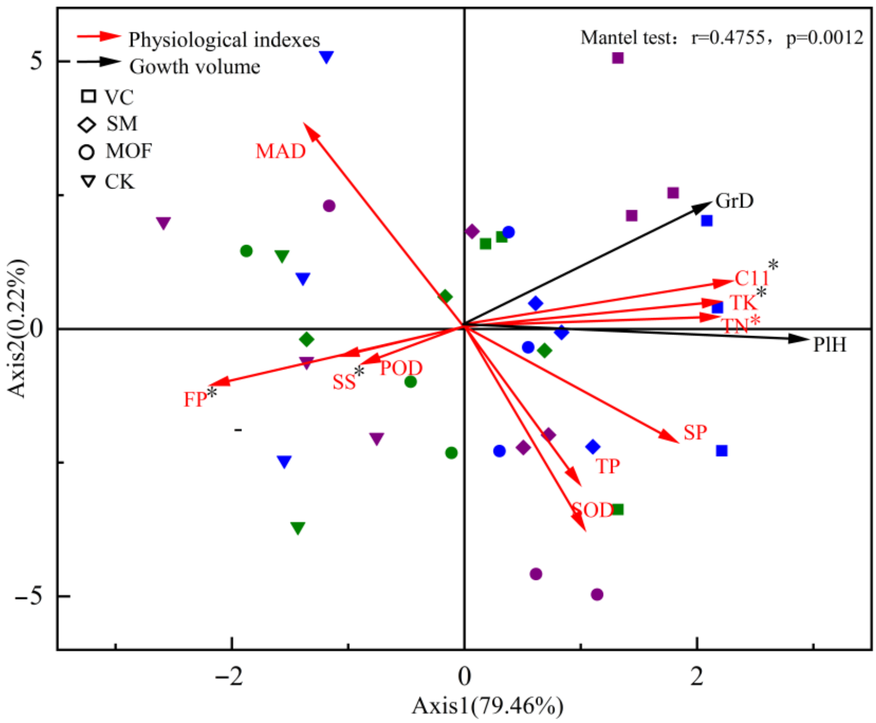


**Figure 7.** Redundancy analysis of growth and physiological indexes of "Qi-Nan" agarwood seedlings under four kinds of fertilization treatments: No fertilizer (CK); vermicompost (VC); sheep manure(SM); microbial organic fertilizer (MOF); plant height (PlH); stem diameter (GrD); chlorophyll (Chl); available nitrogen (TN); available phosphorus (TP); available potassium (TK); superoxide dismutase (SOD); malondialdehyde (MDA); peroxidase (POD); free proline (FP); soluble protein (SP); soluble sugar (SS). * indicates a significant correlation at $p < 0.05$ under mental text. Green in the figure represents the measured value of indicators in November 2021; purple represents the measured value of indicators in December 2022; blue represents the measured value of indicators in May 2022.

The spatial distribution of each fertilization treatment group in the figure shows that the non-fertilization treatment group (CK) is mainly distributed in the second and third quadrants. In contrast, the fertilization treatment group is primarily distributed in the first and fourth quadrants, indicating significant differences in the physiology and nutrient content of "Qi-Nan" agarwood seedlings under fertilization and non-fertilization treatment.

The relationship between each fertilization treatment group and the indices of "Qi-Nan" agarwood seedlings was different. In the CK treatment group, MDA, FP, SP, and POD were more noticeable than other indicators; in the VC treatment group, PlH, GrD, Chl, TK, and TN were more evident than other indices; and in the SM and the MOF treatment groups, SS, TP, and SOD were more evident than other indicators. MDA and FP are the stress resistance indices in plants, and their levels are higher under CK treatment and decrease from left to right along the first ranking axis, indicating that the stress of "Qi-Nan" agarwood seedlings is reduced after the application of bio-organic fertilizer. At the same time, the PlH, GrD, Chl, TK, TN, SS, TP, and SOD indices increased from left to right along the first-ranking axis, indicating that the growth, nutrient content, chlorophyll, and nutrients of "Qi-Nan" agarwood seedlings increased after the application of bio-organic fertilizer.

It can be seen from the angle between the indicators that, in growth indicators, PlH and GrD are positively correlated with Chl, TK, and TN and negatively correlated with MDA, Pro, SP, and POD. In physiological indicators, SP, SOD, and MDA were negatively correlated. In nutrient indicators, TN and TK were positively correlated with each other, while TP and MDA were negatively correlated.

The Mantel test showed that (r: 0.4755, $p = 0.0012$), FP, Chl, TK, and TN, were significantly correlated with plant height and stem diameter ($p \leq 0.05$). It is concluded that FP, Chl, TK, TN, and SS are essential factors affecting the height and stem diameter of "Qi-Nan" agarwood seedlings.

Membership function analysis was used to analyze the physiological and nutrient indices of the "Qi-Nan" agarwood seedlings, and the overall differences of each index under the four fertilization treatments were further evaluated. As shown in Table 4, the ranking of the average membership function values of the four groups is VC > MOF > SM > CK. Thus, VC is the best, and there is little difference between MOF and SM.

**Table 4.** Analysis of the subordination degree of physiological effects of "Qi-Nan" agarwood seedlings treated with different bio-organic fertilizer treatments.

| Index | VC | SM | MOF | CK |
|---|---|---|---|---|
| Plant height | 1.00 | 0.63 | 0.57 | 0.00 |
| Ground diameter | 1.00 | 0.55 | 0.38 | 0.00 |
| Soluble protein | 0.46 | 1.00 | 0.64 | 0.00 |
| Soluble sugar | 0.43 | 0.07 | 1.00 | 0.00 |
| Free proline | 0.00 | 0.46 | 0.55 | 1.00 |
| SOD | 0.10 | 0.30 | 1.00 | 0.00 |
| POD | 0.63 | 1.00 | 0.19 | 0.00 |
| MDA | 0.41 | 0.47 | 0.00 | 1.00 |
| Chlorophyll | 1.00 | 0.57 | 0.55 | 0.00 |
| TN | 1.00 | 0.59 | 0.43 | 0.00 |
| TP | 0.81 | 0.71 | 1.00 | 0.00 |
| TK | 1.00 | 0.16 | 0.49 | 0.00 |
| Mean value | 0.65 | 0.54 | 0.57 | 0.17 |
| Final priorities | 1 | 3 | 2 | 4 |

No fertilizer (CK); Vermicompost (VC); Sheep manure (SM); Microbial organic fertilizer (MOF).

## 5. Discussion

### *5.1. Effect of Different Bio-Organic Fertilizer Treatments on the Growth of "Qi-Nan" Agarwood Seedlings*

The growth status of trees can be reflected by plant height and stem diameter [47]. In this study, it was found that the height and stem diameter of "Qi-Nan" agarwood treated with different bio-organic fertilizers were greater than those of the control group without fertilizer, indicating that the growth of "Qi-Nan" agarwood seedlings was promoted by bio-organic fertilizer. This result is similar to the fertilization research results of olives [48]. With the increase in fertilization time, a growth trend of "slow-fast-slow" was observed, and the growth pattern was in line with the "S" curve. The possible reason is that the root system of the seedlings is weak at the early growth stage, and "Qi-Nan" is stressed by the applied fertilizer, which results in a period of slow seedlings growth. In addition, compared to SM and MOF treatment, the height and diameter of "Qi-Nan" agarwood were more significantly enhanced by VC, with increases of 69.55% and 82.29%, respectively. The reason for this may be that VC is more likely to increase the population of beneficial microorganisms, thus improving soil fertility, which is important for the performance of the field and the plant [49], so the growth of "Qi-Nan" can be more significantly promoted by VC. This result is similar to previous studies on organic fertilizer for cucumber [35], watermelon [50], and other crops. It has also been shown that the growth of *Withania somnifera* [51], okra [52], and other plants can be effectively promoted by VC. These findings are consistent with the experimental results of this paper. The application of VC significantly encourages plant growth and has excellent advantages in plant nutrient supply and soil quality improvement. VC is known as the "king of organic fertilizer" [53,54]. In addition, RDA revealed that FP, chlorophyll, TN, and TK were the most important factors influencing the height and stem diameter of "Qi-Nan" seedlings. Among them, chlorophyll content is not only affected by light intensity but also by soil nitrogen [55], indicating that TN and TK may play relatively vital roles in the cultivation of "Qi-Nan" seedlings.

*5.2. Effect of Different Bio-Organic Fertilizer Treatments on the Physiology of "Qi-Nan" Seedlings*

Determining the levels of soluble sugar, soluble protein, malondialdehyde, free proline, and other indicators helps to understand the physiological and metabolic status of plants [56,57]. Among them, SS and SP are indispensable nutrients in plants, which also play an essential role in maintaining osmoregulation in plants [56]. In this study, the SP content increased first and then decreased with time, which may be due to the cold weather after February; most of the SP accumulated, and consumption fell. This result is similar to the research results of *Erythrophleum fordii* [58] and *Podocarpus macrophyllus* [59]. It is worth noting that previous studies showed that appropriate fertilizer could increase the SS content in plants; for example, the application of bio-organic fertilizer increased the SS content in bananas [60]. Vermicompost at 5 and 10 t/ha significantly increased German chamomile's chlorophyll and soluble sugar content [61]. However, this study found that after applying fertilizer, the SS content of "Qi-Nan" agarwood seedlings decreased continuously, similar to the result of mixed fertilization on *Podocarpus macrophyllus* seedlings [59]. This result may be caused by the following reasons: after the end of fertilization, SOD content in the seedlings of "Qi-Nan" agarwood and the consumption of plant nutrients may be too fast when its activity is high, resulting in the decrease in SS content. In addition, the coming winter weather led to the seedlings storing energy for the winter, so the SS content was the highest in November. FP and MDA are indices of stress resistance in plants [62], and their contents reached the maximum at CK in the control group. From November to February, when the weather turned cold, low-temperature stress increased the accumulation of FP and MDA. However, the FP and MDA contents of "Qi-Nan" agarwood in the fertilization group were still lower than those in the control group, indicating that this situation was alleviated by bio-organic fertilizer, which was consistent with the research results of *Withania somnifera* [51]. Light energy is converted into chemical energy by chlorophyll, and the photosynthetic capacity of plants is directly affected by chlorophyll content [63]. In this study, the chlorophyll l content of "Qi-Nan" agarwood seedlings treated with fertilizer was higher, which indicated the chlorophyll content, photosynthetic performance, and dry matter accumulation of "Qi-Nan" agarwood seedlings was promoted by fertilizer. This result was similar to the effects of fertilization studies on saffron [64] and other crops [65]. Among the fertilizers used, under the treatment of VC, the chlorophyll content of "Qi-Nan" agarwood seedlings was higher. It may be that the root activity and root growth of plants are promoted by VC, so the nitrogen absorption capacity of the plants is thusenhanced [66]. POD and SOD, as protective enzyme systems in plants, are used to remove reactive oxygen species free radicals, relieve membrane lipid peroxidation of cells, and reduce the damage to plants caused by the hostile environment [67]. After the application of bio-organic fertilizer, the contents of POD, FP, MDA, and SS decreased, while the contents of SOD, SP, and chlorophyll increased. This indicates that as the "Qi-Nan" seedlings grew, osmoregulation increased, enhancing photosynthesis and the ability to scavenge reactive oxygen species, which facilitated the physiological metabolism of the seedlings.

*5.3. Effect of Different Bio-Organic Fertilizer Treatments on the Nutrient Accumulation of "Qi-Nan" Agarwood Seedlings*

Plant nutrients are mainly absorbed through the root system, and the leaves in the aboveground part are the main organs used to absorb mineral elements. Leaf nutrition is the auxiliary method of plant nutrition [68]. This experiment showed that the nutrient content of the leaves of "Qi-Nan" agarwood seedlings was significantly increased under the application of bio-organic fertilizer, and the contents of TN, TP, and TK of the seedlings were the best in VC. Relevant studies have shown that compared with other fertilizers, such as pig manure and constant chemical fertilizer, crops' accumulation of nutrient content can be better promoted by VC. For example, the nutrient content and yield of crops such as sugarcane [69] and capsicum [70] were significantly higher after the application of VC than other fertilization treatments. In addition, related experiments were also conducted on narcissus [71], French marigold [72], and other plants. These results were similar to those of the nutrient indicators of

"Qi-Nan" agarwood seedlings. This result is probably because VC is rich in N, P, K, and other nutritional elements. Compared with cow manure, the nitrogen content in VC is 144.3% higher, the phosphorus content is 366.5% higher, and the potassium content is 248.9% higher [73,74]. In other words, VC provides sufficient nutrients for the soil, which is more conducive to the absorption and utilization of nutrients by seedlings plants, especially to meet the needs of nutrients in the later stage of plant growth.

## 6. Conclusions

"Qi-Nan" agarwood is the highest quality and value agarwood on the market. However, no one has studied its suitable fertilization methods thus far. Our research found that the application of bio-organic fertilizer significantly promoted the growth of plant height and stem diameter; increased the contents of SP, SS, chlorophyll, and leaf nutrients; decreased the contents of FP, POD, MDA, and other stress indices; and alleviated the stress caused by low temperatures. RDA analysis showed that the main factors affecting the seedlings' heights and diameters were free proline, chlorophyll, TN, and TP. The VC used in this experiment did contain higher levels of TN and TP, which may have contributed to the accumulation of height and stem diameter of "Qi-Nan" agarwood seedlings. In general, the best growth of "Qi-Nan" seedlings was observed in the VC treatment, followed by the MOF treatment and SM treatment, but the differences were not significant. However, it should be noted that nutrient stress caused by the application of too much fertilizer is a problem that needs to be addressed during the production and fertilization of "Qi-Nan" seedlings. Therefore, different concentrations of base fertilizer and topdressing fertilizer can be designed in follow-up research to expand the application value of the experiment results.

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