Chapter

Cultivation Practice of Chinese Medicinal Herbs

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Abstract

An innovative cultivation technique for Chinese medicinal herbs had been practiced in China, which led a new road for medicinal herbs production without input of chemical fertilizer and chemical pesticides. The organic practice was based on the principle of biodiversity for pest control. An example of *Panax notoginseng* (Burk.) F. H. Chen was chosen for explaining cultivation technology under forest. The key technologies for *P. notoginseng* cultivation under forest include forest land selection, land tillage, seedling breeding and transplanting, and on-farm organic management. These technologies can standardize herbs production in large-scale under forest, and the quality and safety of *P. notoginseng* can be effectively improved without applying chemical pesticides and chemical fertilizer in the production process.

Keywords: Chinese herbs, cultivation under forest, biodiversity for pest control, organic production

1. Introduction

For thousands of years, Chinese medicinal herbs were collected from forested mountains, which faded away with the demands of human healthy [1]. People have to choose artificial cultivation in the field and adopted high-yield method for its planting. However, high-yield method betrayed the characteristic of medicinal herb, which caused medical effectiveness and quality to decrease deeply [2]. We explore a new road for medicinal herb cultivation under forest without chemical fertilizer and pesticide. It is based on the principle of biodiversity for pest control.

2. Background and cultivation technology of Chinese medicinal herbs under forest

2.1 The background of Chinese medicinal herbs

Chinese herbs are the important raw material for pharmaceutical production and the material basis for the inheritance and development of traditional Chinese medicine (TCM) for thousands of years. It is also a strategic resource related to the national economy and people's livelihood. As an important support of economic development in many poor areas, it plays a positive role in increasing farmers' income [3]. The clinical value of TCM has progressively been appreciated by the public at home and abroad, particularly the effect shown in the COVID-19 prevention and control, which has demonstrated its unique efficacy and role [4]. With the increasing global population and the complication of disease types, the demand for Chinese herbal medicine continues to rise. It shows a vigorous development trend in the Chinese herbal medicine industry. Historically, the supply of Chinese herbal medicine commodity mainly depends on wild plant resources [1]. Nevertheless, with the soaring demand for medicinal herbs, natural medicinal herbs have been mined out, and artificial farmland cultivation has gradually been adopted. Since the 1950s, China has vigorously developed the production of Chinese herbal medicine, from introduction and trial planting to commercial planting to base cultivation. The planting industry of Chinese herbs has experienced four periods: the germination period, the development period, the growth period, and the prosperity period [2]. However, some problems occurred with the gradual increase in the planting scale and yield of Chinese herbs that require urgent solutions.

First, the quality of Chinese herbs is unstable. At present, the circulation market of Chinese herbs is mixed with different varieties, including wild varieties, semi-wild varieties, wild cultivation, cultivated varieties, genuine land varieties, introduced varieties from different places, etc. [5]. The multichannel sources of TCM lead to its uneven internal quality, which not only increases quality security control difficulty of TCM, but also has a negative impact on the processing of TCM decoction pieces and TCM preparations [6]. The quality of Chinese herbs is affected by various objective elements.

Second, the problem of pesticide residues and excessive heavy metals is prominent. As the market demand for TCM continues to increase, wild medicinal materials have been hollowed out, and its yield has been unable to meet the demand, so most of the medicinal materials have to be cultivated artificially. In the process of cultivation, the high-yield mode of crops was copied for Chinese herbs, and a large number of chemical fertilizers, expansion, and other chemicals were applied, resulting in high yield but low medicinal effective component. The quality of medicinal herbs declined, and the efficacy of Chinese herbs was generally doubted [7]. Moreover, in the large-scale agricultural production of traditional Chinese medicine, pest became severe, and farmers have to apply chemical pesticides in order to guarantee the output, but the lack of scientific management of the field use of pesticides leads to the existence of different degrees of pesticide residue in medicinal herbs [8]. Exogenous harmful residues of medicinal herbs mainly include pesticide residues, heavy metals, polycyclic aromatic hydrocarbons (PAHs), and others [9]. Unreasonable use of pesticides leads to uneven quality of TCM decoction pieces and cannot guarantee their safety [10]. The quality problems of pesticide residues and excessive heavy metals restrict the international development of traditional medicine [11]. The 2020 edition of the Chinese Pharmacopeia has more stringent restrictions on pesticide usage; besides, the European Union, Japan, and South Korea impose severe restrictions on pesticide residues and heavy metals. The Chinese herbal medicine industry will face more serious challenges.

Third, the continuous cultivation disorder of Chinese herbs is serious, and most areas producing genuine medicinal herbs face the risk of having no land to grow [12]. With the increasing demand for Chinese medicine materials, the types and scale of artificial cultivation of that are increasing, but the problem of continuous cropping in cultivation is becoming increasingly prominent, which seriously affects the normal growth and medicinal value and brings great trouble to the development of Chinese medicine [13]. Most of the TCM materials copy the agricultural high-yield mode; however, the reality is that crop varieties have adapted to the high-yield mode after thousands of years of genetic transformation, while the research and development of medicinal herbs are insufficient to support the high-yield mode. Therefore, the application of agricultural high-yield mode has caused major problems such as high yield but low quality, excessive pesticide residues, and continuous cropping obstacles in the production of Chinese herbs. About 70% of the root Chinese medicinal herbs are slow-growing perennial herbs that take many years to reach reproductive maturity; these plants usually experience different degrees of replant obstacles [12, 14]. In addition, most medicinal herbs are not fertilizer-resistant, and soil eutrophication leads to serious obstacles to continuous cultivation. A crop of cultivated land cannot be replanted for several decades, which leads to the serious phenomenon of deforestation for planting Chinese herbs, and most authentic producing areas face the embarrassing situation of having no land to plant, which seriously restricts the sustainable development of the Chinese herbal medicine industry [15, 16].

Around these problems, agricultural scientists in China took the research on large varieties of Chinese herbs such as *Panax notoginseng*, *Panax ginseng*, and *Dendrobium nobile* as a breakthrough point to explore and form a rule that follows the natural growth and development of medicinal herbs, that is, to use under-forest resources to make medicinal materials return to wild forests, and establish the planting mode based on the efficacy of medicinal materials as the first principle, and gradually form a set of perfect theoretical and technical systems to solve the problem of quality decline of Chinese herbs from the source, which provides a strong technical guarantee for the healthy development of the Chinese herbal medicine industry. Theoretical basis of medicinal herbs under forest [17–23].

In the agroforestry system, agroforestry intercropping mode can not only significantly improve the microclimate conditions under forests by reducing soil erosion and wind speed, but also improve soil fertility and crop yield [24]. This pattern can make full use of natural resources and improve the efficiency of land use. Currently, the agroforestry intercropping mode has been successful in walnut/mung bean [25], poplar/soybean [26], jujube/cotton [27], etc. Therefore, the agroforestry intercropping model provides an effective and scientific way to alleviate the shortage of land resources and realize sustainable utilization.

2.2 Cultivation technology of Chinese medicinal herbs under forest

A typical example for explaining planting technology is *Panax notoginseng* (Burk.) F. H. Chen cultivated under forest [28]. *P. notoginseng* belongs to Panax genus, the Araliaceae family. It is one of the most famous Chinese traditional medicinal herbs. It is a rare medicinal herb unique in China and has the functions of promoting blood circulation, dispersing blood stasis, reducing swelling, and relieving pain. As a medicinal herb, *P. notoginseng* has been used for hundreds of years. It was first recorded in Compendium of Materia Medica by Li Shizhen in the Ming Dynasty. The main components of *P. notoginseng* are polysaccharides and saponins and also contain a small amount of flavonoids and volatile oil, as well as trace metal elements and other substances, among which saponins are the main medicinal components. The cultivation of *P. notoginseng* requires very special environmental conditions, including altitude, climate, and soil. Yunnan Province and Guangxi Province are the main planting areas, among which Yunnan province accounts for more than 98% of the total planting area in China [16]. Moreover, Yunnan Province is the authentic production area of *P. notoginseng*, and its yield and quality are better than other those in production areas.

Due to its high medicinal and economic value, *P. notoginseng* has attracted more and more attention. However, pesticides residues and heavy metals overuse, continuous cultivation obstacles, and unstable quality, etc., threaten the development of Chinese medicines. Facing these problems, we had explored the roads under the lead of outstanding phytopathologist Zhu youyong from Yunnan Agricultural University. We have been engaged in the research and promotion of ecological cultivation of high-quality *P. notoginseng* for many years. The previous study of our group showed that Yunnan Pine and Simao Pine have a high canopy density, which is suitable for the shade-demand growth of *P. notoginseng*. The volatiles of pine trees have the effect of pest control. Organic matter formed by the degradation of pine needles is also beneficial to the healthy growth of *P. notoginseng*. In 2015, our group carried out the test for *P. notoginseng* cultivation under the forest of Simao Pine at the altitude of 1500 m ~ 1900 m in the Zhutang Town of Lancang County in Yunnan Province. After 2 years, this innovative cultivation mode achieved success. The technical standards for *P. notoginseng* cultivation under forest were established, and three local standards were issued on June 11, 2020, which promoted poverty reduction in China and was conducive to the rational use of forest resources.

The key technologies for *P. notoginseng* cultivation under forest include forest land selection, land tillage, seedling breeding and transplanting, and on-farm organic management. Using these key technologies can realize the standardization and large-scale production for *P. notoginseng* planting under forest, and the quality and safety of *P. notoginseng* can be effectively improved without applying chemical pesticides and chemical fertilizer in the production process.

2.2.1 Forest land selection

When carrying out the under-forest organic planting for *P. notoginseng*, artificial forestlands and the tree species that have no negative effects on the growth of *P. notoginseng* such as Yunnan Pine, Simao Pine, Huashan pine, Chinese fir, etc., could be selected. The forest canopy density should be 0.7 ~ 0.9 for the growth of *P. notoginseng*, and forest land with slope ≤ 25° could be able to be used for planting because of ecological conservation and.

In addition, the suitable soil for *P. notoginseng* is sandy loam soil or loam soil, and the soil pollutant content should meet the requirements of national standard GB15618. Selected forest land should have not been planted with any crop for at least 8 years so that it meets organic production.

2.2.2 Under-forest land tillage

1. Land preparation. Weeds and small shrubs under the forest should be moved and dead branches and leaves should be cleared. It is important that weeds and shrubs in non-planting areas should be retained as much as possible.

2. Plowing. The soil in the planting area was plowed 2 ~ 3 times with a depth of 20 cm ~ 30 cm by manual or small rotary tillers.

3. Ridging. The ridge direction should be determined according to the natural terrain or tree arrangement: flat land should be ridged according to the direction

of forest tree planting. The slope land should be ridged along the contour line. The ridge surface is arranged in a circular arc, and the distance between the ridge and the tree root is greater than 20 cm. The ridge height is adjusted according to the slope. When the slope is greater than 20°, the ridge height is about 30 cm. When the slope is less than 20°, the ridge height is about 40 cm.

4. Setting up the drainage ditches. Drainage ditch is necessary in rainy season, and it is set up according to different slopes and terrain and leaves a drainage outlet every 15 m ~ 20 m length of the ridge. The drain outlet should be cleaned timely to prevent tree branches or soil accumulation from obstructing the drainage.

2.2.3 Seedling and transplanting under forest

The seedling preparation of *P. notoginseng* for under-forest cultivation includes two steps: seedling and transplanting.

1. Seedling. Seedling refers to sowing healthy *P. notoginseng* seeds on the ridge surface under the forest and using the soil under the forest environment for seedling cultivation. Healthy and high plumpness of seeds should be selected for under-forest seedling, and according to the relevant provisions of GB/T 19630, biological sources that can inhibit the pathogen should be selected to treat the seeds. The sowing time is controlled from mid-November to January of the following year. The sowing method is single seed sowing on demand, with raw spacing not less than 5 cm and the sowing depth is 1.0 cm ~ 2.0 cm. The seeding was covered with fine soil with a thickness of 3 ~ 5 cm, the soil surface was then covered with pine needles with a thickness of 2 ~ 5 cm. Watering after sowing to ensure that the water fully permeates the soil.

2. Seedling transplanting. Seedling transplanting refers to transplanting healthy *P. notoginseng* seedlings to the planting ridge under the forest for 2–3 years of growth. Seedlings with a weight per plant at least 1.5 g, main root diameter of at least 0.9 cm, dormant bud diameter of at least 0.4 cm, and no pesticide residue after pesticide residues detection are transplanted. Seedlings should be transplanted on sunny days during the period of early December to late January of the following year. The transplanting was carried out according to the standards of the (10 cm ~ 15 cm) × (10 cm ~ 15 cm) in row spacing and 3 cm ~ 5 cm in depth. Seedlings should be discharged horizontally and arranged on a line. Make the dormant buds head down and cover the soil with a thickness of 3 cm ~ 5 cm while transplanting. Then the planting ridge surface is evenly and tightly covered with pine needles with a thickness of 2 cm ~ 5 cm. Watering after transplanting to ensure that the water fully permeates the soil.

2.2.4 On-farm management of under-forest P. notoginseng

1. Water management. The water management of under-forest *P. notoginseng* is divided into four stages: ① Before seedling: The soil should be given a good soaking timely after sowing, and the humidity of ridge surface should be observed regularly. It should be watered well in time when the soil moisture content is less than 20%. In the case of too much rainfall, pay attention to drainage and waterlogging prevention.② Seedling germination: After seedling emergence, a small

amount of water should be irrigated many times to ensure that the soil moisture content is not less than 20%. The germination period of *P. notoginseng* overlaps with the dry season in Yunnan. The soil under the forest is loose and the water retention capacity is poor. *P. notoginseng* is easy to dehydrate, which leads to pests and root rot. Therefore, attention must be paid to soil moisture management in production to avoid drought. ③ Rainy season: After entering the rainy season, simple rain shelters should be constructed to avoid excessive soil moisture. When the soil moisture content is less than 20%, the rain-avoidance film can be lifted to accept natural rain showers until the soil is soaked. ④ End of the rainy season and dormant period: The soil also needs to be moist during this period. Soil water shortage will aggravate the problems of root pests and root rot, resulting in a decrease in seedling emergence rate in the next year and affecting the yield of under-forest *P. notoginseng*.

2. Nutrient management. The organic matter under the forest is rich, and no chemical synthetic fertilizer is allowed to be applied throughout the production process of *P. notoginseng*, only light and water management are needed. If the organic content of the soil is less than 2%, organic fertilizer can be appropriately supplemented after the emergence of *P. notoginseng* leaves. The types and selection of organic fertilizer should meet the relevant provisions of GB / T 19630.

3. Ecological prevention and control for diseases. There are several main diseases on *P. notoginseng* in the forest: ① Leaf diseases: rounded spot disease, black spot, powdery mildew, anthracnose disease, etc. ② Root diseases: root rot disease, root-knot nematode disease, vertical blight, quenching disease, etc.

   We conducted the protocol for diseases control: ① Elimination of disease residues. The diseased plants found under the forest should be cleared in time to prevent the spread of pathogens; ② physical prevention and control. Before the rainy season, timely cleaning of the drainage ditch in the plantation for drainage to avoid root rot. Moreover, by building a simple rain shelter on the ridge surface under the forest, it can regulate environmental humidity and play a role in physical prevention and control of diseases. ③ Biological control. Under-forest bases should adopt products such as bio-control bacteria, bio-derived fungicides, and plant elicitors specified in GB/T 19630 for disease prevention or treatment, and the use of chemical pesticides should be strictly prohibited.

4. Weed management. Weeds that affect the growth of *P. notoginseng* on the ridge surfaces (such as *Eupatorium adenophorum)* should be removed promptly, and damage to the *P. notoginseng* root system should be avoided during the removal. Weed that has a shallow root system and does not affect the growth of *P. notoginseng* can be retained to transfer pests.

5. Prevention and control of rat harm. To use natural enemies such as weasel, snakes, etc., to control forest rats; according to the specific forest environment, rat traps, electronic mouse expeller, and other equipment are set up in the rat gathering area or channel to prevent rat harm.

6. Plantation clearance. During the growth of *P. notoginseng*, especially in spring and autumn, there are many dead branches under the forest. When clearing the

plantation, it is necessary to remove the dead branches on the ridge surface, and remove weeds and excessive pine needles that are not conducive to the growth of *P. notoginseng*. From late December to February of the following year is the dormancy period of under-forest *P. notoginseng*, the fallen dead branches and leaves in the forest should be completely removed to keep the ridge clean and healthy, and the dead branches and leaves of aboveground parts of *P. notoginseng* should be cut (cut at >5 cm away from the pine needle).

2.3 Principles of cultivation practice for Chinese medicinal herbs under the forest

Ecological balance principle. Traditional Chinese herbs are mostly grown in the natural ecological system, with high biodiversity, natural growth of medicinal materials, light pests and diseases, high efficacy, and sufficient medicinal power. However, in the high-yield cultivation mode of farmland, the variety of medicinal materials is single, the biodiversity of farmland is reduced, the ecosystem is unbalanced, and the harm of harmful organisms is intensified, resulting in the decrease in quality and safety of medicinal materials. It is an effective way to reduce the harm of diseases and insects and improve the quality of medicinal materials by using the natural law of interspecies mutual restraint. Based on the principle of under-forest biodiversity interaction and ecological balance, the planting of shade-growing Chinese herbs and forest system were effectively combined to construct an efficient, stable, and diverse agroforestry system. On this basis, according to the regularity of occurrence and development of Chinese herbal medicine diseases, the rain-shelter cultivation system of under-forest Chinese herbal medicine can also be constructed. Rain-shelter cultivation can create microclimate conditions that are not conducive to the development of pathogen to achieve physical prevention and control of the plant pathogen [18].

Habitat coupling principle. The cultivation of under-forest Chinese herbs makes full use of the coupling characteristics of forest habitats (light, temperature, water, fertilizer, etc.) and the growth characteristics of Chinese herbal medicines and realizes the genuine and high-quality production of Chinese herbal medicines. The main principles of habitat coupling include: ① light coupling principle. Chinese herbs suitable for under-forest cultivation are shade plants originating from the lower forest vegetation, which are sensitive to strong light [29, 30]. Inappropriate light will affect the growth and quality of Chinese herbs. Under the condition of conventional farmland planting, it is necessary to artificially build a shading shed to simulate its suitable light environment, but it is difficult to simulate to achieve its optimal growth conditions. Under-forest planting of Chinese herbs only uses the light microenvironment naturally formed under forest vegetation, which is more conducive to the growth and quality formation of Chinese herbs [31, 32]. Thermal coupling principle. Chinese herbal medicines are usually sensitive to heat. The artificial shading shed built in farmland has poor buffering capacity for heat, which often leads to heat injury or freezing damage [33, 34]. Due to the shade of trees, the lower layer of forest vegetation has strong buffering capacity for temperature, which can effectively alleviate the stress caused by the fierce change of temperature on the growth of medicinal herbs [35, 36]. ③ Water coupling principle. Forest-shade Chinese herbs growing under the forest have wet-like characteristics. The dry environment is not conducive to their growth. The forest environment can improve the microclimate, slow down the flow of air, and reduce water evaporation, thereby improving the distribution and utilization of water. ④ Nutrient coupling principle. The soil nutrient balance is the material basis for maintaining the primary and secondary metabolic balance of medicinal herbs. At present, the input of organic fertilizer is less and less in the farmland system, and the use of a large number of chemical fertilizers makes the problems of soil acidification and salinization increasingly prominent, resulting in the decline of efficacy, diseases and pests, and continuous cropping obstacles [37, 38]. In the agroforestry system, the roots and leaves of trees can provide a large amount of organic matter for the soil. The decomposition and accumulation of these organic matters can improve the fertility of the soil and provide sufficient nutrient supply for the healthy growth of Chinese herbs [39]. The soil rich in organic matter can also increase the diversity of microorganisms, promote the growth of beneficial microorganisms, and improve the stability of the soil ecosystem and antiviral ability [40]; in addition, understory soils rich in organic matter and microorganisms also produce some disease-resistant substances, which induce plant resistance and effectively reduce the occurrence of diseases [41].

The under-forest ecosystem has rich biodiversity, such as plant diversity, animal diversity, insect diversity, and microbial diversity. Plant–plant, plant-microorganism, plant–animal (insect), and other organisms in the under-forest system form a stable community through complex mutual generation and restriction relationship among the various organisms, which has strong ability to resist natural disasters.

3. Mechanism of cultivation practice for Chinese medicinal herbs under forest

Without chemical fertilizers and pesticides, *P. notoginseng* has been successfully cultivated in forest ecosystems. It was based on the principle of biodiversity for pest control. Most of *P. notoginseng* was cultivated in coniferous forest [*Pinus kesiya* var. *langbianensis*, *Pinus yunnanensis*, *Pinus armandii Franch*. and *Cunninghamia lanceolata* (Lamb.) Hook] (**Figure 1**). The cultivation of *P. notoginseng* under-forest conditions is successful due to the effective use of (i) native environment and biodiversity and (ii) stimulatory allelopathic interactions within the forest system (**Figure 2**).

Forest environment: *P. notoginseng* is shade-loving plant that thrives well under low light intensity and 18–25°C temperature. When the light intensity is >30% of sunlight and temperature is over 30°C, the growth environment becomes harsh for *P. notoginseng*. The environment in the lower layers of forests provides desirable shade and temperature for P. *notoginseng* growth. Compared with traditional cultivation model, this forest cultivation model does not use polyethylene net or straw to provide shade, which greatly reduces the cost. Besides, the leaf litter acts as cover, keeps the soil moist, and maintains suitable temperature for *P. notoginseng* growth.

Soil rich in organic matter and microbes: Long-term decomposition of forest residues improves the soil quality. We found that the physicochemical properties of coniferous forest soil meet the requirements of *P. notoginseng* (pH: 5.5–6.5; electrical conductivity: 60–120 μS cm^{-1}; nutrient contents: 250–360 mg kg^{-1} available N, 10–25 mg kg^{-1} available P, 100–260 mg kg^{-1} available K, and 80–120 g kg^{-1} soil organic matter). Such soil provides adequate nutrients for *P. notoginseng* growth and development of strong root system (**Figure 1**).

Forest soils are generally rich in microorganisms, which reduces the root rot disease and promotes the plant growth by increasing the availability of nutrients. Our laboratory experiments showed that application of microbiome from the forest soil into sick soil improves the germination and growth of *P. notoginseng* and alleviates the soil-borne diseases. Bacteria with strong antifungal activity (*Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Burkholderia* spp. etc.), against *P. notoginseng* root

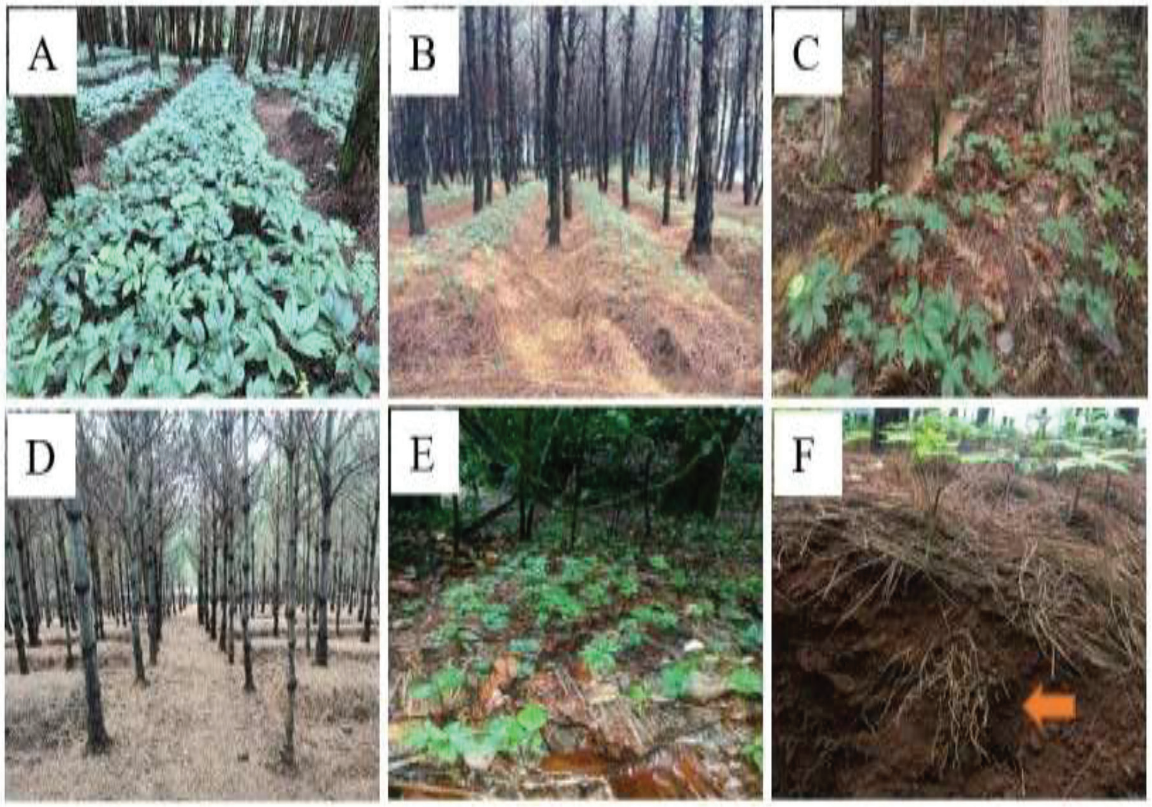

Figure 1.
Cultivation of P. notoginseng *under forest conditions. A.* Pinuskesiya var. langbianensis *forest; B.* Pinus yunnanensis *forest; C.* Cunninghamia lanceolata *(Lamb.) Hook. forest; D.* Pinus armandii *Franch. forest; E. Broadleaf forest; F. The strong roots of* P. notoginseng *in forest soil.*

rot pathogens, have been isolated and identified from forest soil. Besides, the atmospheric nitrogen-fixing microbes (*Bradyrhizobium* spp., *Rhizobium* spp., *Frankia* spp. etc.) are also abundant in forest soils [42].

Allelopathic interactions in forest. Cultivation of *P. notoginseng* under natural forest conditions uses the allelopathy to stimulate the plant growth and prevents the pests and diseases infestation than in monoculture in farmland or greenhouses. The possible pathways for the release of allelochemicals into the environment are: (i) emission of volatile organic compounds (VOCs) from different plant tissue, (ii) leaching from leaves by rain or dew, (iii) decay of plant residues, and (iv) root exudation.

The VOCs of pine tree terpenes (including α-pinene, β-pinene, camphene, etc.) stimulated the growth and reduced the pests and diseases of *P. notoginseng*. Riedlmeier et al. [43] reported that monoterpenes, particularly pinenes, could enhance the systemic acquired resistance in *Arabidopsis thaliana* to the pathogen *Pseudomonas syringae*. Likewise, our results showed that some VOCs from pine needles induce the resistance of *P. notoginseng* to leaf black spot disease (caused by *Alternaria tenuis* Nees). In addition, α- terpineol and terpinen-4-ol are antifungal to growth of fungal pathogens (*Fusarium oxysporum*, *Fusarium solani*, *Cylindrocarpon destructans*, *Phytophthora cactorum*, and *A. tenuis* Nees) (unpublished). It is also observed that leachates from pine needles stimulated the growth and biomass of *P. notoginseng*. Terpenes are one kind of VOCs, from the herbivore damaged plants that attract the predators or parasitoids to attack herbivores [44]. Aldrich et al. [45] reported that an artificial pheromone containing α-terpineol and Terpinen-4-ol emitted from pine could attract the adult predaceous spined soldier bugs, *Podisus maculiventris* (Say).

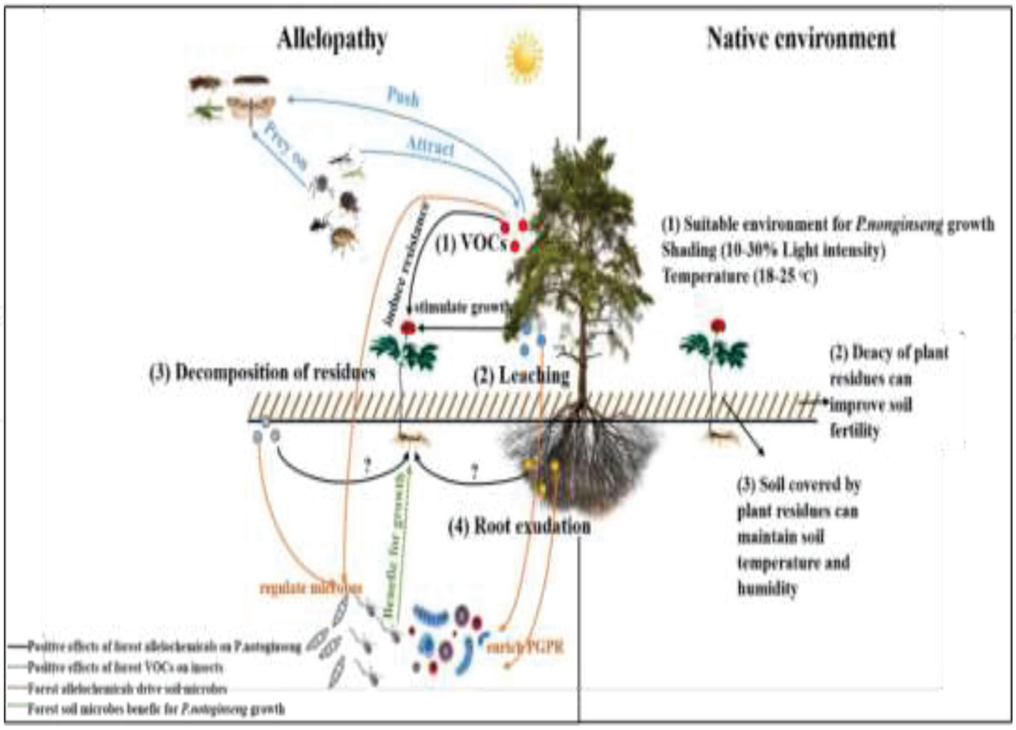

Figure 2.
Mechanisms involved in the cultivation of P. notoginseng *under natural forest conditions [42].*

This confirms that the VOCs from coniferous forests play a positive allelopathic role in pest control.

Cao et al. [46] investigated the diversity of arthropods on *P. notoginseng*, planted in *P. kesiya* var. *langbianensis* forest. The data showed that there were a total of 40 species of arthropods from 33 families, of which the natural enemies were 40%. The Shannon-wiener diversity index of natural enemy subcommunity is significantly higher than the pest subcommunity. Obviously, a high biodiversity of arthropods significantly reduces the damage caused by pests in *P. notoginseng*.

4. Diseases occurrence and control of *P. notoginseng* under forest

Diseases are more sever in Chinese medicinal herbs than in other crops because they grow for longer time and are easily threatened by diseases. However, diseases have not been prevalent in the forest, which contributes to the measures of cultivation under forest described above.

4.1 Round spot disease occurrence characteristics

We had observed the disease from 2016 in the *P. notoginseng* plantations in Lancang County, Pu′er City, Yunnan Province. In rainy season, round, water-soaked spots on the leaves of *P. notoginseng* appeared when it was exposed in rain. Round or nearly round watery spots on leaves are initial symptoms of round spot disease. The lesion appeared transparent when it was viewed in the sun compared with healthy leaf tissue. Then the brown infection spots were observed in the center of the disease spots (**Figure 3A**). Eventually, the lesions gradually expanded, turned brown or grayish brown, and had "wheel marks" (**Figure 3B**). Later, white powdery conidial piles formed on the lesion surface (**Figure 3C**). Finally, multiple lesions merged, causing

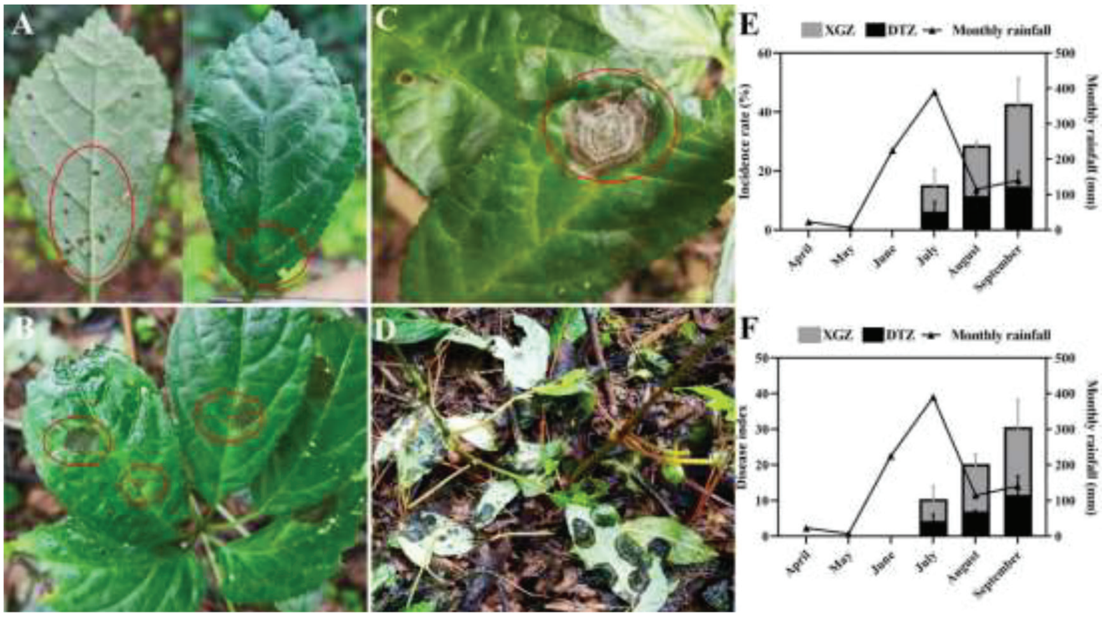

Figure 3.
Symptoms, incidence, and disease index of round spot disease. (A) Watery spots on P. notoginseng *leaves. (B) Watery spots expand to form black wheel-like lesions. (C) Gray white conidia piles form on the lesions. (D) Leaves fall off in the late stage of the disease. (E) Relationship between incidence rate of round spot disease and monthly rainfall in DTZ and XGZ. (F) Relationship between disease index of round spot disease and monthly rainfall in DTZ and XGZ. XGZ: Xiaoguangzha* P. notoginseng *plantation. DTZ: Datangzi* P. notoginseng *plantation.*

the leaves to rot and fall off during rainfall (**Figure 3D**). Based on these characteristics of the disease spots, the disease was preliminarily identified as round spot disease.

The incidence of round spot disease in Lancang County was investigated and monitored. *P. notoginseng* begins to germinate from March to April. From March to June, no round spot disease symptoms were observed (**Figure 3E** and **F**). As of July, round spot disease started to occur. Round spot disease incidence rose in August and September. Lancang County has a subtropical wet summer and dry winter mountain monsoon climate, with distinct rainy and dry seasons. Generally, rainfall is limited in April and May, but begins to increase as of June. **Figure 3E** and **F** shows that round spot disease started to occur after the rainy season.

4.2 Isolation and identification of the causal pathogen of round spot disease

The pathogen causing round spot disease was isolated by single-spore isolation. In total, 30 pathogen strains were isolated from 30 diseased leaves. On PDA, colonies were round, villous, and varied in color from red to gray (**Figure 4A** and **B**). The strains did not produce conidia on PDA; they required water to induce conidia production. Conidiophores produced by thickened hyphae were colorless and bent (**Figure 4F** and **G**). Conidia were solitary, colorless, transparent, and long-arched, with multiple diaphragms (**Figure 4C** and **D**). The tail of the conidia gradually became thinner and the apical cells appeared to be truncated. A long, narrow, accessory filament with septa grew on the side of the apical cell. Notably, thin cilia were observed at the tail of the conidium and the top of the accessory filament (**Figure 4E**). The total length of conidia, including the accessory filament and two cilia, was 171.83–492.92 μm (average, 349.74 μm) (n = 200). Their width, measured at the widest part in the middle of the conidia, was 6.24–13.05 μm (average,

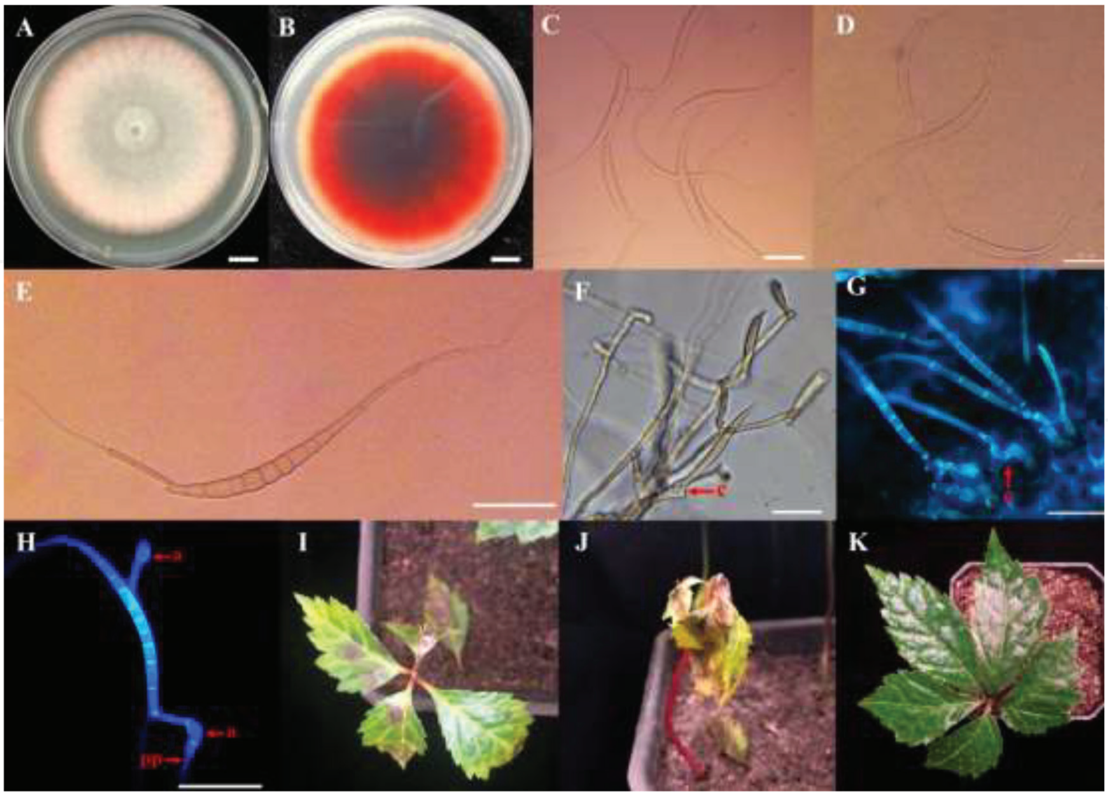

Figure 4.
Morphological characteristics of Mycocentrospora acerina*. (A, B) mycelial colony morphology of* M. acerina *cultured on PDA medium for 7 days at 20°C. (C–E) morphological characteristics of conidia. (F, G) morphological characteristics of conidiophores and conidia. The conidiophores and conidia in panel G were stained with calcofluor white. c: Conidiophore. (H) Conidia on the leaves of* P. notoginseng *germinated to produce an appressorium and penetration peg. A: Appressorium, pp.: Penetration peg. (I) Symptoms on* P. notoginseng *leaves 7 days after inoculation of a conidial suspension. (J) Symptoms on* P. notoginseng *leaves 14 days after inoculation of a conidial suspension. (K)* P. notoginseng *leaves remained healthy for 7 days when being mock-inoculated with sterile water. Scale bars = 10 mm in A–B, scale bars = 50 μm in C–H.*

8.61 μm). Based on the morphology and conidial characteristics, the pathogen was identified as *M. acerina* [47].

The pathogenicity of 10 isolated strains was determined. Seven days post inoculation, all inoculated leaves showed similar lesions, and the symptoms were identical to those in the field (**Figure 4I** and **J**). The control plants remained healthy (**Figure 4K**). The diseased leaves in each treatment were selected for pathogen isolation, and the pathogen was reidentified as *M. acerina*.

4.3 Effects of temperature and wetness duration on conidial germination *in vitro*

Conidial germination was evaluated and observed at all 11 temperatures, except 32°C (**Figure 5A**). Conidia did not germinate within 2 h at all temperatures. At 4, 8, 14, 18, 20, 22, 24, and 28°C, germination started at 4 h. At 6 h, the germination rates at 14, 18, 20, 22, and 24°C all exceeded 30%. At 18 h, the germination rates at 14, 18, 20, 22, and 24°C all exceeded 90%. The highest germination rate was observed at 20°C (97.16%). At 24 h, all conidia at 18, 20, and 22°C had germinated. The germination rate exceeded 90% at 8, 14, and 24°C. Thus, temperatures above 28°C and below 4°C are not conducive to conidial germination, with the optimal temperature for germination between 14 and 22°C.

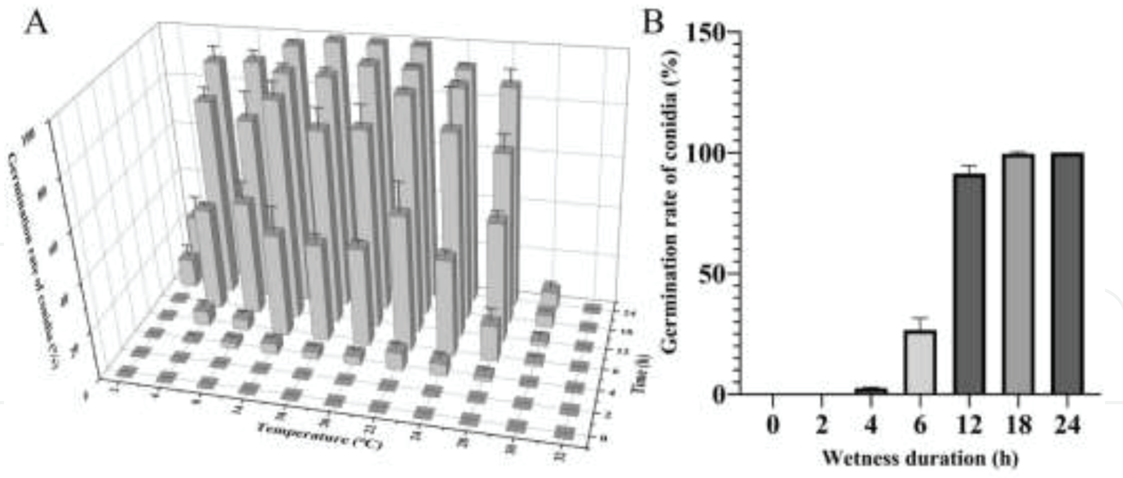

Figure 5.
Effects of temperature and wetness duration on conidial germination in vitro. (A) Average germination rate of conidia at 11 different temperatures and different time points. (B) Average germination rate of conidia under seven different wetness durations.

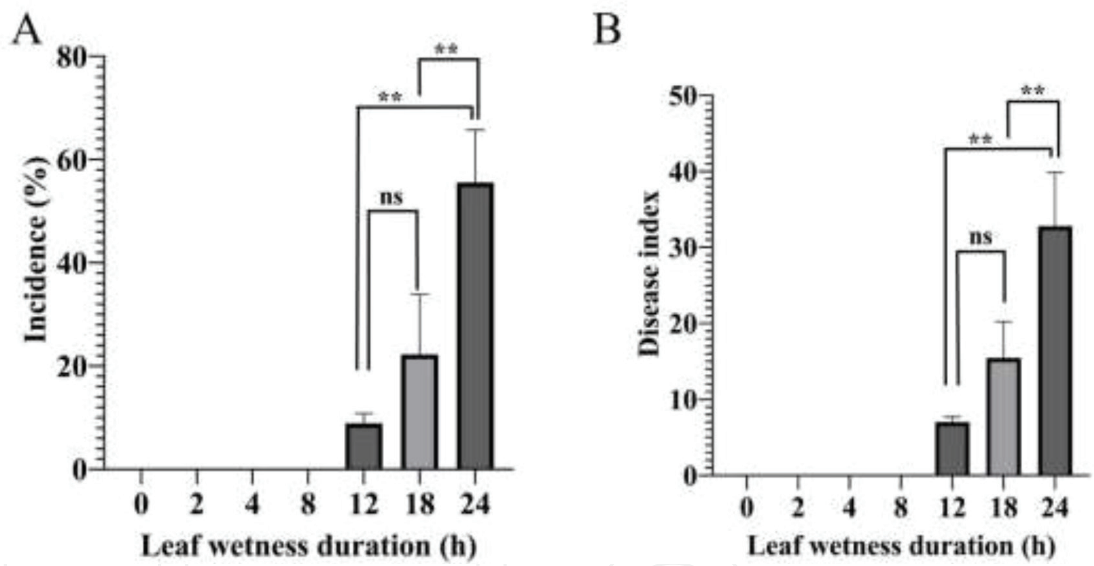

Figure 6.
*Effects of different leaf wetness durations on the occurrence of round spot disease. (A) Incidence and (B) disease index of round spot disease for different leaf wetness durations. *P < 0.05, **P < 0.01; n = 3, ns: no significant difference.*

Free water is a key factor for conidial germination. The effect of leaf wetness duration was first investigated *in vitro*. When the wetness duration was less than 2 h, conidia could not germinate (**Figure 5B**). Between 2 and 24 h, the germination rate increased with increasing wetness duration. All conidia were germinated when a free water film was present for 24 h. Even at 99% relative humidity, in the absence of free water, the conidia did not germinate.

4.4 Effect of *P. notoginseng* leaf wetness duration on conidial infection

To evaluate the effect of leaf wetness duration on conidial infection, in the greenhouse experiment, round spot disease incidence was investigated 10 days after inoculation. When the leaf wetness duration was shorter than or equal to 8 h, no disease spots appeared on the *P. notoginseng* leaf surface. When the leaf wetness duration was longer than or equal to 12 h, the incidence and disease index of round spot disease increased according to the leaf wetness duration (**Figure 4A** and **B**). Round spot disease incidence and disease index at a wetness duration of 24 h were 55.56% and 32.78, respectively, and were significantly higher than those at a wetness duration of 12 h and 18 h (**Figure 6**).

4.5 Infection process of *M. acerina* conidia on *P. notoginseng* leaves

The different infection stages of *M. acerina* conidia on *P. notoginseng* leaves were observed and photographed. For microscopic observation, we used fluorescence staining with calcofluor white. Under excitation with ultraviolet light, conidia and hyphae emit blue fluorescence. The development of conidia on *P. notoginseng* leaves between 0 and 18 h is shown in **Figure 7**. At 6 h, the conidia had grown multiple primary hyphae from cells at different locations. Between 6 and 12 h, the primary hyphae continued to elongate, and at 12 h, some of the hyphal ends were expanded to form an appressorium. At 18 h, lesions had appeared on the leaf surface, which indicated that hyphae had invaded the *P. notoginseng* leaves. The development of conidia on *P. notoginseng* leaves between 24 and 96 h is shown in **Figure 6**. By 24 h, the lesion area had expanded and the lesion became conspicuous, with an appressorium in the center. By 48 h, the lesion center had turned yellow and hyphae on the leaf surface no longer elongated. At 72 h, the lesion was further enlarged and the color of the lesion center changed from yellow to brown. At 96 h, multiple conidia appeared on the lesion surface. The conidia were formed by the mycelium growing and developing in the lesions. The lifecycle of *M. acerina*, from conidial inoculation to the production of conidia, is completed in 4 days (**Figure 8**).

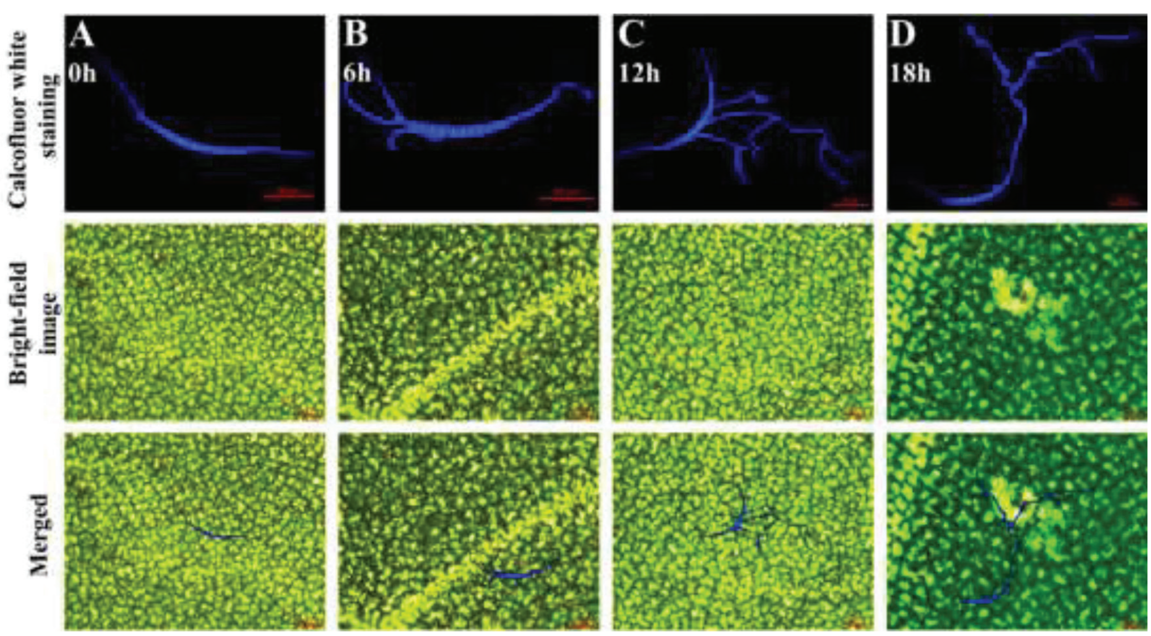

Figure 7.
Development of Mycocentrospora acerina *conidia on* P. notoginseng *leaves. (A–D) conidia were observed on the leaves of P. notoginseng at 0, 6, 12, and 18 h, respectively. The blue color shows* Mycocentrospora acerina *conidia and hyphae stained with calcofluor white stain. The bright-field image shows* P. notoginseng *leaf epidermal cells, and the merged image shows the germination and invasion of* Mycocentrospora acerina *conidia on* P. notoginseng *leaves. Bar = 50 μm.*

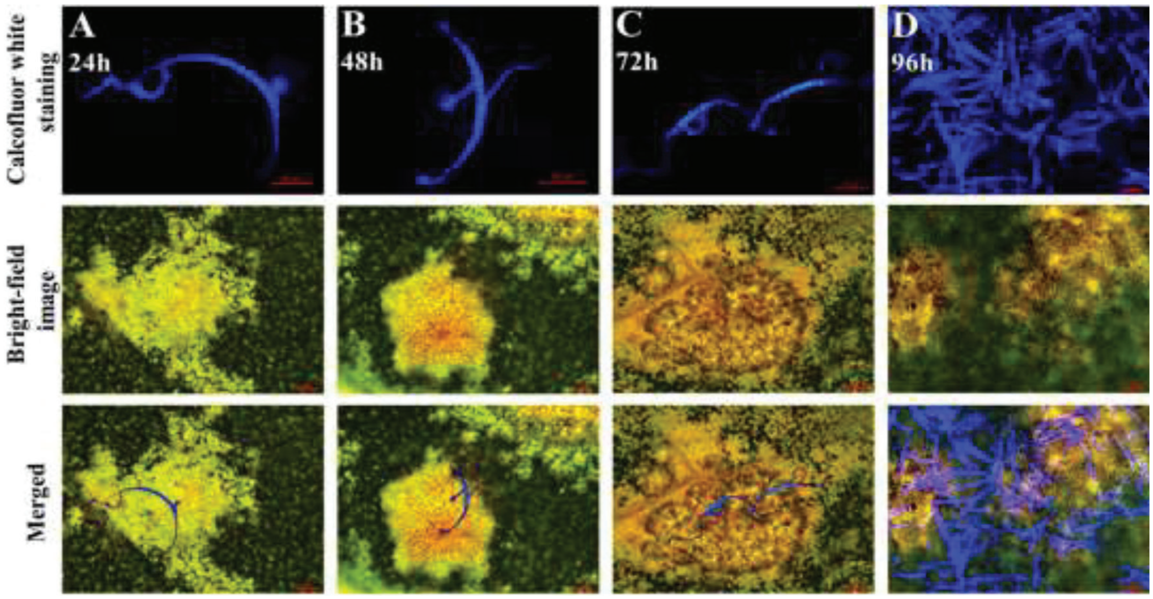

Figure 8.
Development of Mycocentrospora acerina *conidia on* P. notoginseng *leaves. (A–D) conidia were observed on the leaves of* P. notoginseng *at 24, 48, 72, and 96 h, respectively. The blue color shows* Mycocentrospora acerina *conidia and hyphae stained with calcofluor white stain. The bright-field image shows P. notoginseng leaf epidermal cells, and the merged image shows the germination and invasion of* M. acerina *conidia on* P. notoginseng *leaves. Bar = 50 µm.*

4.6 Control of round spot disease by rain-shelter cultivation

In 2020, the rainy season in Lancang County began in June. At the end of June, a rain shelter was built in the *P. notoginseng* field and an open-field control treatment was set up simultaneously. On July 10, the first round spot disease incidence survey was conducted. Neither sheltered nor non-sheltered *P. notoginseng* plants developed the disease (**Figure 9**). However, on July 20, round spot disease started to occur in some areas of the open field. In contrast, the sheltered plants remained healthy. Subsequently, round spot disease began to spread in the open field. By August 10, the average round spot disease incidence in the open field was 18.07%, and the disease index was 8.89. However, the incidence in sheltered plants was still 0.00%. Over time, the disease incidence in the open field increased. As of September 30, the incidence

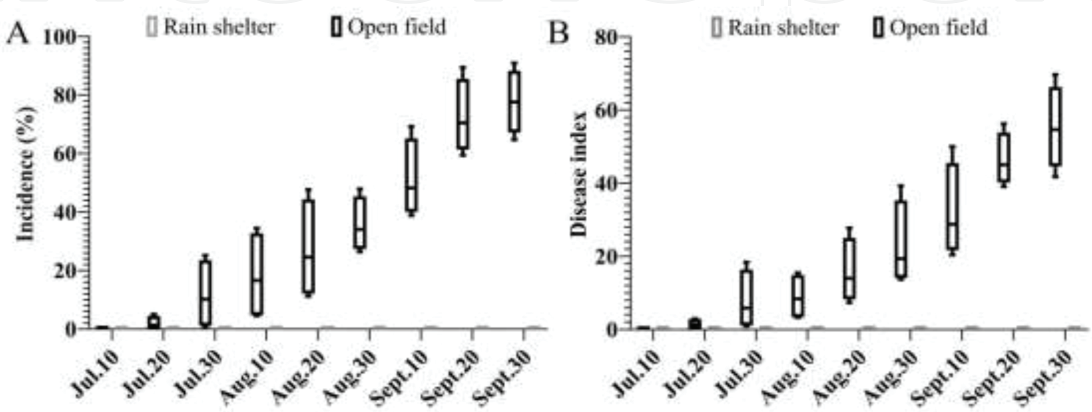

Figure 9.
Effect of rain-shelter cultivation on the incidence rate and disease index of round spot disease. (A) Incidence and (B) disease index of round spot disease in rain-shelter and open-field cultivation.

Figure 10.
Effects of rain-shelter cultivation and open-field cultivation on the occurrence of round spot disease. (A) P. notoginseng *plants grown under shelter cultivation remained healthy. (B) Round spot disease occurred in open-field cultivation.*

had reached 77.70%, covering nearly the entire open-field treatment area. However, sheltered plants still remained healthy (**Figure 10**). These data indicated that rain-shelter cultivation has good preventive and control effects on the occurrence of round spot disease, with an average preventive effect of 100%.

5. Conclusion

Cultivation practice of Chinese herbs under forest is a promising technique in the future. It does not occupy arable land, no chemical fertilizer and pesticides, which leads a healthy route for medicinal herbs production.

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