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# Study on the use of Imazalil to continuous cropping obstacle of *Ganoderma lucidum* caused by *Xylogone ganodermophthora*

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## Abstract

**Background** The *Xylogone ganodermophthora* is a pathogenic bacterium that poses a significant challenge to the continuous cultivation of the *Ganoderma lucidum* fungus. This study aims to identify and investigate specific agents for the effective prevention and control of *X. ganodermophthora*, establishing a theoretical foundation for overcoming this persistent challenge in *G. lucidum* cultivation.

**Results** Using different *G. lucidum* soil as materials to study the presence of *X. ganodermophthora* in the soil. Additionally, the plate confrontation test was employed to investigate the impact of *X. ganodermophthora* on *G. lucidum* growth. The impact of physical factors and antibacterial agents on pathogenic bacteria was successfully carried out, with a further exploration of the effectiveness of field control. PCR amplification experiment and sequencing analysis verified that *X. ganodermophthora* existed in *G. lucidum* continuous cropping obstacle soil. This pathogenic bacteria has a significant inhibitory effect on the growth of *G. lucidum*, with an inhibition rate of up to 52.23%. High temperature, low temperature, light and other physical factors have no obvious inhibitory effect on this pathogen. Further investigation revealed that specific drugs, such as low concentrations (10 μL/mL) of Acticide DB20 and Imazalil, could effectively inhibit *X. ganodermophthora* growth in *G. lucidum*. Among them, Imazalil has a notable inhibitory effect on the growth of *X. ganodermophthora*.

**Conclusions** Indoor toxicity test and field control results showed that Imazalil could effectively control the growth of pathogen *X. ganodermophthora* in *G. lucidum* continuous cropping obstacle, and promote the growth of *G. lucidum*.

**Keywords** *Xylogone ganodermophthora*, *Ganoderma lucidum*, Continuous cropping obstacle, Fungicide screening, Imazalil

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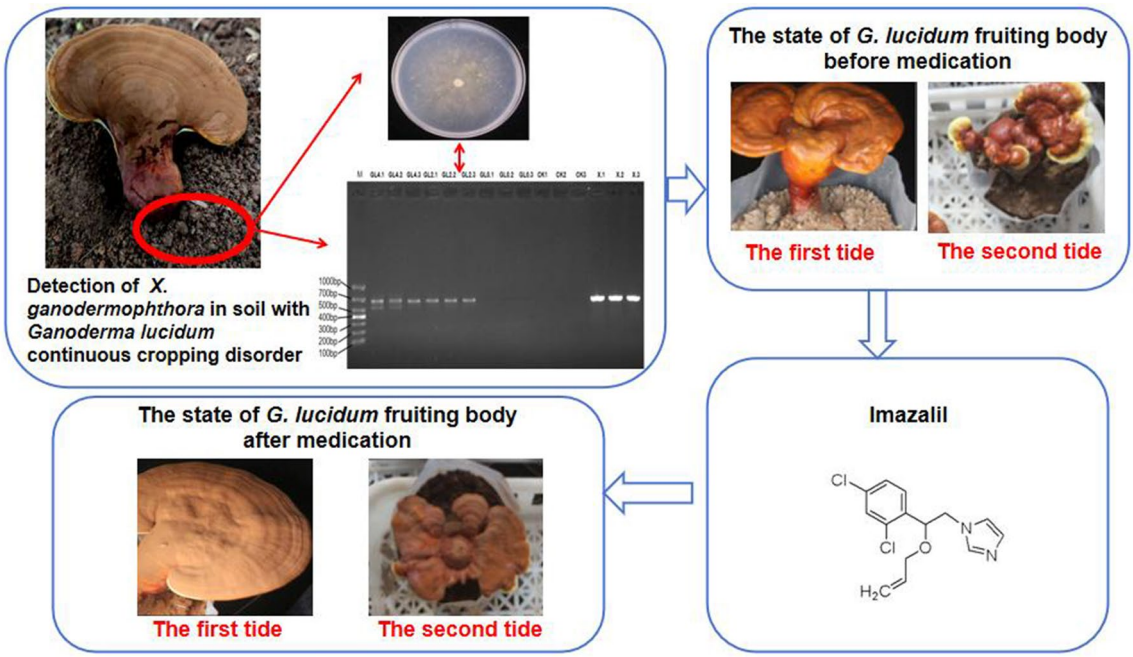
**Graphical Abstract**




## Introduction

*Ganoderma lucidum*, also known as fairy grass, belongs to Ganoderma of Basidiomycetes, Polyporaceae. It is a kind of large fungi with both medicinal and edible functions [1]. Historical texts such as *Shen Nong Ben Cao Jing*, *Re-repair of politics and history of the standby Materia Medica,* and *Ben Cao Gang Mu* have documented the positive effects of *G. lucidum* on mental energy, blood production, mind relaxation, and the treatment of deafness. *G. lucidum* has been extensively utilized in China, Japan, Korea, the Philippines, Malaysia, Singapore, and Indonesia [2, 3]. Following the inclusion of *G. lucidum* fruiting bodies as legal Chinese medicinal materials in *Chinese Pharmacopoeia* [2, 3], *G. lucidum* was included in *American Herbal Pharmacopoeia and Therapeutic Compendium* in 2010 [4]. However, there is a limited supply of wild *G. lucidum*, meaning that the natural supply falls short of the increasing market demand. Therefore, the artificial cultivation of *G. lucidum* industry has also emerged. Artificial cultivation has caused the production of *G. lucidum* to increase year by year. Based on data, from 2010 to 2020, there was a significant increase in the production of *G. lucidum* in China, with the output increasing from 91,200 tons to 190,000 tons. Industrial cultivation of *G. lucidum* has contributed significantly to the growth of the industry. Currently, there are various *G. lucidum* processing products available in the market, including *G. lucidum* spore powder and granules [5].

In the process of planting *G. lucidum* in farmland, a common issue that arises after 2–3 years is a continuous cropping obstacle, "*Ganoderma lucidum* continuous cropping obstacle" refers to the phenomenon that *G. lucidum* is continuously planted in the same soil area during the artificial cultivation process, and even under normal cultivation and management conditions, the growth becomes weak, the pests and diseases intensify, and the yield decreases [6]. This phenomenon is influenced by various factors, such as shifts in soil biochemical factors, changes in soil microbial population characteristics, autotoxicity of crop root products, and the proliferation of certain pathogenic bacteria [7, 8]. Currently, soil disinfection and sterilization methods can somewhat alleviate the continuous cropping obstacle for *G. lucidum*. However, it can also lead to soil hardening, fertility decline, and weakened resistance [9]. Therefore, there is an urgent need to identify a viable solution for the ongoing production of *G. lucidum* to allow for continued growth of the *G. lucidum* industry.

Liu et al. [10] isolated and purified a pure culture of ascomycete when studying continuous cropping

obstacle of *G. lucidum* grown on basswood, and determined it as *X. ganodermophthora* by morphological observation and molecular identification. Since Kang et al. [11] first discovered this fungus on *G. lucidum* yellow rot and confirmed its pathogenicity to *G. lucidum* [11], Tong et al. [12] found this fungus in associated fungi of *G. lucidum* in Cambodia [12]. Typical symptoms include the internal tissues at the base of the *G. lucidum* or the tissues within the basswood that have grown *G. lucidum* and been infected turn yellow. No or only a few fruiting bodies formed, or the pileus growing on the infected section of wood was malformed. Yellow rot can cause yield reduction, limit the continuous cultivation in the same location, and it requires the cultivation site to be changed on the third year following two years of inoculation. The above results indicate that there is a concomitant relationship between *X. ganodermophthora* and the growth of *G. lucidum*, and it may be one of the pathogens of *Ganoderma lucidum* continuous cropping obstacle, and may transmit pathogenicity through cultivated soil.

The purpose of this experiment is to verify whether *X. ganodermophthora* exists in *G. lucidum* continuous cropping soil, explore its influence on the growth of ten *G. lucidum* varieties, and at the same time to prevent it, to find a new way to solve *G. lucidum* continuous cropping obstacles.

## Materials and methods

### Soil samples and conservation

Samples were taken at four locations on January 13, 2021. CK was taken from random soil near the first dining hall of Fujian Agriculture and Forestry University (26.093534° N, 119.242990° E), GL0 was taken from the wild soil near the Organic Ganoderma Expo Park in Nanping City, Pucheng County, Fujian Province, GL2 and GL4 (27.928986° N, 118.526909° E) were taken from the soil planted with *Ganoderma lucidum* for 2 years and *G. lucidum* for 4 years in the Organic Ganoderma Expo Park. The straight-line distance between CK and GL2 is about 221.2 km, GL2 and GL4 are located in different greenhouses in the same park, the straight-line distance is about 113 m, and the straight-line distance between GL4 and GL0 is about 1.4 km. Four experimental soils, each with 3 biological replicates, each sample about 100 g, were stored at − 20℃.

### Strain samples and source

Ten species of *Ganoderma* genus, *Ganoderma multipileum* (Strain Number: CBS 128579), *Ganodermats tsugae* (Strain Number: BCRC 36821), *Ganoderma lucidum* (Strain Number: CGMCC 5.0026), *Ganoderma sinense* (Strain Number: BNCC143276), *Ganoderma leucocontextum 2*, *Ganoderma leucocontextum 1*, *Ganoderma leucocontextum Y2019, Ganoderma resinaceum*, *sporeless cultivar of Ganoderma lingzhi,* and *Ganoderma leucocontextum 1905* were preserved at the Mycological Research Center of Fujian Agriculture and Forestry University, Fujian, China. The strain of *X. ganodermophthora* (Strain Number: UAMH 10320) used in this work originated from the Academy of Agricultural Sciences in Lishui City.

### Reagents

We selected 9 different chemicals, namely Hachemical CPH (Abbreviated as CPH), Acticide DB20 (DB20), Antioxidant HAP (HAP) were purchased from Heng'an Fine Chemical Co., Ltd. Triforine (TRF), Vinclozolin (VIN) were purchased from Sigma. Procymidone (PRC) was purchased from Sumitomo Chemical Shanghai Co., Ltd. Lime (LIM) was purchased from Xinyu Huihui Industrial Co., Ltd. Fumigation (FUM) was purchased from Fuzhou Liqiang Disinfectant Co., Ltd. Imazalil (IMA) was purchased from Jiangxi Heyi Chemical Co., Ltd.

### Instruments

HWS12 thermostatic water bath form Shanghai Yiheng Technology Co., LTD., China. PCR Instrument form Hangzhou Langi Scientific Instrument Co., LTD. EPS300 electrophoresis apparatus form Shanghai Tianneng Technology Co., LTD.

### Soil DNA extraction and detection

*G. lucidum* soil DNA was extracted from different groups, including the CK group, GL0 group, GL2 group, and GL4 group, following the instructions provided by the soil DNA extraction kit (OMEGA). There were four groups, each group repeated three times. The concentration and purity were determined using a nucleic acid microanalyzer.

### Primer design and PCR amplification

The MH327528 gene sequence in GenBank as well as the Primer Premier 6 software were used to design specific primers. Forward primer: jkj-f:5′-GCGATAAGTAAT GCGAATTG-3′, Reverse primer: jkj-r:5′-CTCCAGAGC GAGATGATG-3′. Primers were synthesized by Beijing Tsingke Biotech Co., Ltd. The following PCR amplification reactions were prepared: 12.5 μL 2×Master Mix, 2.5 μL forward primer, 2.5 μL reverse primer, 7.5 μL DNA template, and 25 μL dd$H_2O$. The following thermocycler conditions were used: 95 °C for 4 min; 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min (32 cycles); 72 °C for

10 min, and stored at 4 °C. PCR amplification products were analyzed by gel electrophoresis on a 2% (*w*/*v*) agarose gel.

#### The inhibitory effect of *X. ganodermophthora* on the growth of *G. lucidum* mycelium

Set *X. ganodermophthora* confrontation group and *G. lucidum* control group. *G. lucidum* control group was inoculated *G. lucidum* only on the PDA plate, in the confrontation group, *X. ganodermophthora* was inoculated 3 cm away from *G. lucidum*. Next, colony diameter was measured and the suppressive impact of *X. ganodermophthora* was measured to determine the inhibition rate on *G. lucidum* mycelium growth.

$$\text{Inhibition rate} = \left[\frac{(\text{Diameter of colony in control group} - \text{Diameter of colony in confrontation group})}{(\text{Diameter of colony in control group} - \text{Diameter of inoculated block})}\right] \times 100\%$$

#### Effects of physical factors on *X. ganodermophthora* growth

(i) Effect of temperature on growth and development of *X. ganodermophthora* hyphae: 8 mm diameter pathogen block was attached to PDA culture dish with hyphal side down, and the culture temperature gradient was set at 20, 25, 28, 32, 35, 40, 45, 50, 55, 60, 65, 70 ℃.

(ii) Effect of pH on growth and development of *X. ganodermophthora* hyphae: pH of the medium was adjusted to 8 different grades (2, 3, 4, 5, 6, 7, 8, 9, 10, 11) with 0.1 mol/L hydrochloric acid (HCl) and 0.1 mol/L sodium hydroxide (NaOH) solution before sterilization. Inoculate an 8 mm diameter pathogen block into the center of the dish. Subsequently, the dishes were maintained at 25 °C in darkness for 5 days.

(iii) Effects of illumination on growth and development of *X. ganodermophthor* hyphae: The lighting parameters of the constant-temperature incubator were adjusted to three settings: full light, full darkness, and a 12-h cycle of alternating light and dark periods. PDA dishes inoculated with pathogenic bacteria (8 mm in diameter) were placed in incubators with three light modes and incubated at 20 °C for 7 days. Each of the aforementioned treatments were replicated in triplicate.

#### Indoor toxicity measurement

Indoor toxicity was determined using the growth rate method. Three types of concentration gradients are set for each agent in Table 1. The pathogens were inoculated into PDA medium and a control group was set up. Record the growth rate of the colonies in a 25 °C incubator. The inhibition rate of mycelium growth was determined by indoor mycelium growth rate inhibition method.

#### Field testing of efficacy

Following analysis of data for the indoor screening of control agents and careful consideration of the costs, Imazalil was identified as a possible candidate for *X. ganodermophthora* growth inhibition. Six experimental groups were established consisting of the following groups: blank control, low-dose control, medium-dose control, tie-back test group, low-dose experimental group, and a medium-dose experimental group. Each group contained 10 fungus packs.

The fungus packs were then positioned within a greenhouse covered by a shading net that provided 90% shade. Proper ventilation was ensured, the carbon dioxide concentration was maintained at the same level as the surrounding atmosphere, and air humidity was maintained at approximately 70%. Upon opening each fungus pack, we ensured that it was covered with a layer of exposed soil (2–3 cm in thickness). The blank control group, low-dose control group, and high-dose control group were treated with 20 mL of water every Wednesday. Conversely, the tie-back test group, low-dose experimental group, and high-dose experimental group were given 20 mL of pathogenic bacterial liquid. Additionally, the blank control group and tie-back test group received 10 mL of water every Sunday. Low-dose control group and low-dose experimental group received low-dose medicine 10 mL every Sunday. Similarly, the medium-dose control group and medium-dose experimental group received 10 mL of

**Table 1** The dosage of 9 fungicides in test reagent

| Fungicides | Dose | | |
|---|---|---|---|
| | Low dose | Medium dose | High dose |
| CPH | 10 μL/mL | 25 μL/mL | 50 μL/mL |
| DB20 | 10 μL/mL | 25 μL/mL | 50 μL/mL |
| HAP | 10 μL/mL | 25 μL/mL | 50 μL/mL |
| PRC | 10 μg/mL | 50 μg/mL | 100 μg/mL |
| TRF | 10 μg/mL | 30 μg/mL | 60 μg/mL |
| LIM | 10 μg/mL | 50 μg/mL | 250 μg/mL |
| FUM | 10 μg/mL | 25 μg/mL | 50 μg/mL |
| VIN | 10 μg/mL | 50 μg/mL | 250 μg/mL |
| IMA | 10 μL/mL | 50 μL/mL | 100 μL/mL |

medium-dose treatment every Sunday. The *G. lucidum* was collected three weeks post-experiment, and many agronomic characteristics were assessed, including the yield of *G. lucidum* per bag, *G. lucidum* per pack, and the drying rate.

### Statistical analyses

Data were subjected to one-way analysis of variance (ANOVA), and the mean values indicating statistical significance were compared by Duncan's multiple-range test using SPSS 25. These data are all expressed as the mean ± standard deviation. $P < 0.05$ were considered to be statistically significant.

## Results and discussion

### Detection of *X. ganodermophthora* in soil utilized for continuous cultivation of *G. lucidum*

Previous studies [10–12] have provided evidence that *X. ganodermophthora* is a pathogenic fungus that infects and hinders the growth of *G. lucidum*. As shown in Fig. 1. Results showed that soil DNA of CK and GL0 groups could not amplify bands, but soil DNA of GL2 and GL4 groups could amplify bands. After sequencing and Genbank alignment analysis, the band sequence was highly homologous to *X. ganodermophthora* in *Xylogone*, indicating that *X. ganodermophthora* existed in continuous cropping soil, and *X. ganodermophthora* was related to continuous cropping obstacle of *G. lucidum*.

### Experimental investigation into the impact of pathogenic bacteria on the growth inhibition of *G. lucidum* during continuous cultivation

Figure 2 shows that on the third day of the confronting culture, the hyphae of *G. lucidum* and *G. sinense* started to make contact with the hyphae of *X. ganodermophthora.* By the fifth day, the hyphae of ten species of *G. lucidum* had been in contact with the *X. ganodermophthora* hyphae (Table 2). By calculating the inhibition rate of pathogenic fungus hyphae on *G. lucidum* hyphae in 5 days, it was found that *X. ganodermophthora* grows extremely fast. It has significant inhibition effect on mycelium growth of 10 different *G. lucidum* strains, more than half of the tested *G. lucidum* strains showed an inhibition rate over 40%. Among them, the inhibition rate of *Ganoderma leucocontextum 1* is 52.63%. Taken together, these data clearly showed that *X. ganodermophthora* inhibits the growth of *G. lucidum* strains.

### Impact of physical conditions on *X. ganodermophthora* proliferation

As shown in Fig. 3, in terms of growth temperature, the growth temperature of *G. lucidum* mycelium ranges from 4 to 35 °C, with an optimal temperature of 24–28 °C. Beyond this temperature range, the mycelium

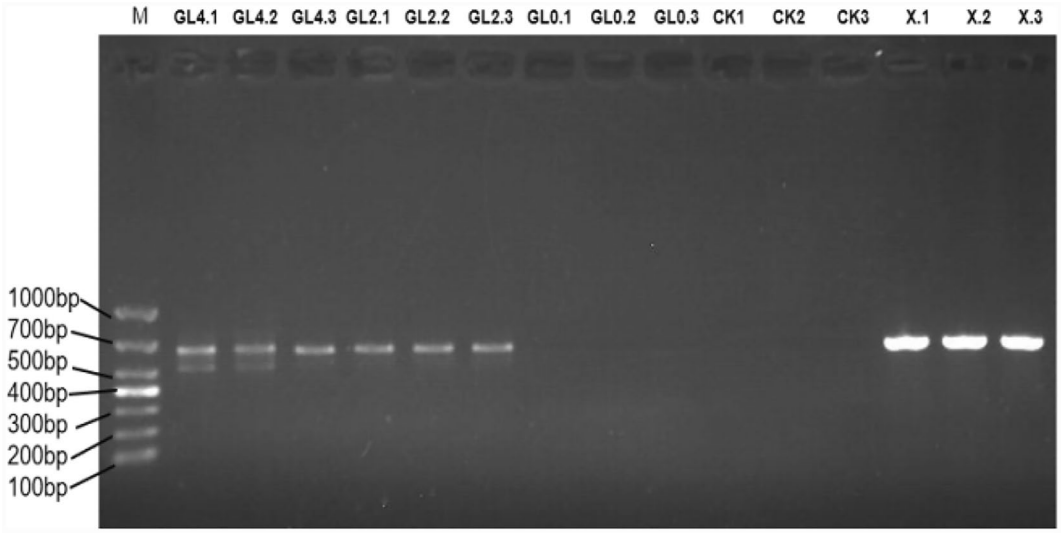



**Fig. 1** Molecular identification of *X. ganodermophthora* in Four Soil Samples. (M) is Marker, 1 kb plus DNA Ladder. In GL4.1–GL4.3, GL4 represents the soil planted with *G. lucidum* for four years, and 1–3 represents three replicates. (GL2.1–GL2.3), GL2 represents the soil planted with *G. lucidum* for two years, and 1–3 represents three repeats. (GL0.1–GL0.3), GL0 represents the soil (wild soil) near the planting place of *G. lucidum*, and 1–3 represents three replicates. (CK)stands for the soil without *G. lucidum*, and 1–3 stands for three replicates. (X) stands for *X. ganodermophthora*, and 1–3 stands for three repetitions

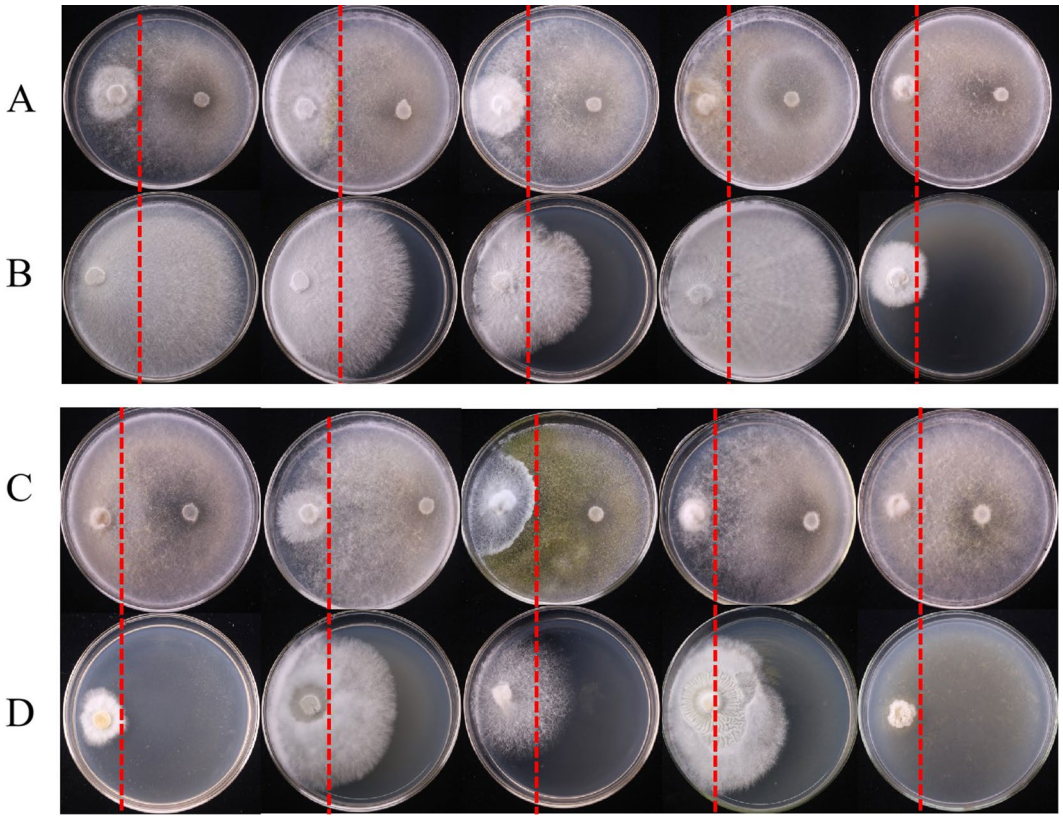



**Fig. 2** Mycelia's growth inhibition of pathogenic on *G. lucidum* by disk diffusion, **A**, **C** treatment group (The fungus block on the left is *G. lucidum* strain, and the fungus block on the right is *X. ganodermophthora*), **B**, **D**: control group; strains of figure **A** and **B**, from left to right, are *G. multipileum, G. tsugae, G. lucidum, G. sinense and G. leucocontextum 2,* respectively; strains of figure **C** and D, from left to right, are *G. leucocontextum 1, G. resinaceum, sporeless cultivar of G. lingzh, G. sinense Y2019* and *G. leucocontextum 1905,* respectively

**Table 2** Mycelia's growth inhibition of pathogenic on *G. lucidum*

| Strains | Date | | |
|---|---|---|---|
| | Colony diameter of *G. lucidum* in control group (cm) | Colony diameter of *G. lucidum* in treatment group (cm) | Inhibition rate (%) |
| *Ganoderma multipileum* | 4.07 ± 0.21 | 2.60 ± 0.10 | 44.90 |
| *Ganodermats tsugae* | 5.10 ± 0.36 | 3.17 ± 0.31 | 44.96 |
| *Ganoderma lucidum* | 4.63 ± 0.06 | 2.80 ± 0.36 | 47.83 |
| *Ganoderma sinense* | 3.37 ± 0.12 | 2.37 ± 0.15 | 38.96 |
| *Ganoderma leucocontextum 2* | 2.00 ± 0.10 | 1.60 ± 0.21 | 33.33 |
| *Ganoderma leucocontextum 1* | 1.43 ± 0.12 | 1.10 ± 0.10 | 52.63 |
| *Ganoderma resinaceum* | 2.57 ± 0.42 | 2.20 ± 0.35 | 20.75 |
| *sporeless cultivar of Ganoderma lingzhi* | 3.97 ± 0.06 | 3.03 ± 0.06 | 29.47 |
| *Ganoderma leucocontextum Y2019* | 2.03 ± 0.38 | 1.67 ± 0.15 | 29.73 |
| *Ganoderma leucocontextum 1905* | 1.50 ± 0.00 | 1.17 ± 0.15 | 47.62 |

stops growing or exhibits abnormal growth or even death. However, pathogenic bacteria can maintain rapid growth at 25–32 °C, with an optimal growth temperature of 28 °C, which is the same temperature for optimal growth of *G. lucidum* mycelium. Regarding pH, different mycelium varieties grown on PDA plate culture medium display significant differences, what they have in common is that they can grow in the range

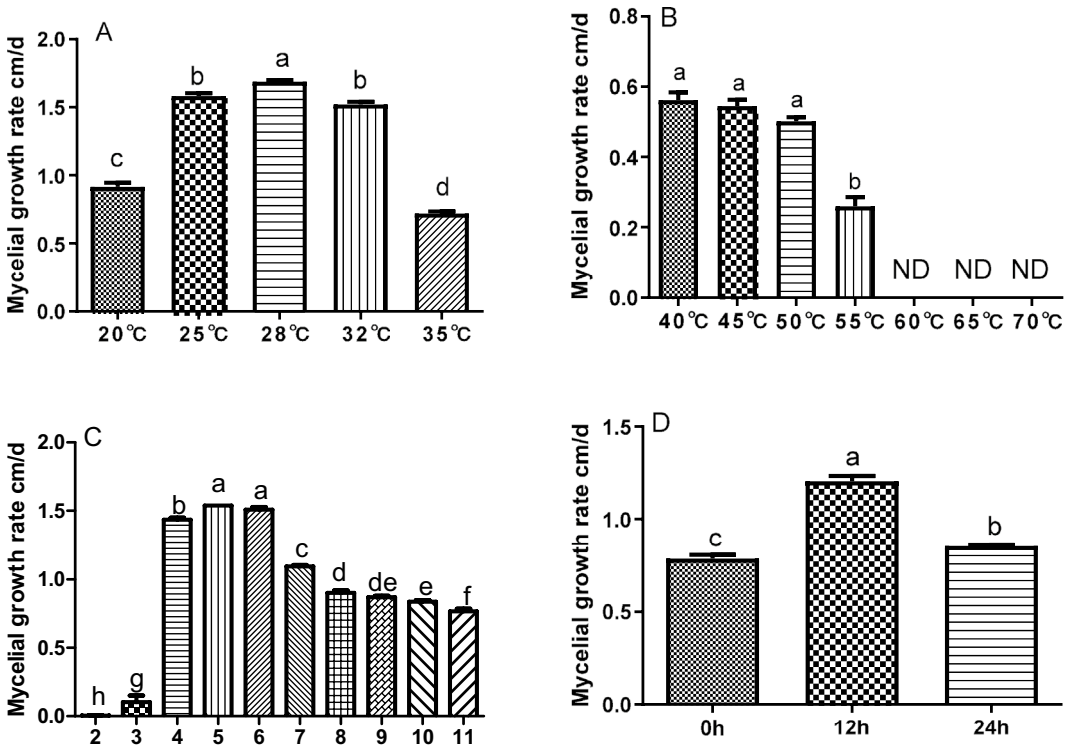



**Fig. 3** Biological characteristics of *X. ganodermophthora*. **A** Mycelial of growth rate of *X. ganodermophthora* in different temperature. **B** Mycelial of growth rate of *X. ganodermophthora* in different lethal temperature. **C** Mycelial of growth rate of *X. ganodermophthora* in different pH. **D** Mycelial of growth rate of *X. ganodermophthora* in different illumination duration

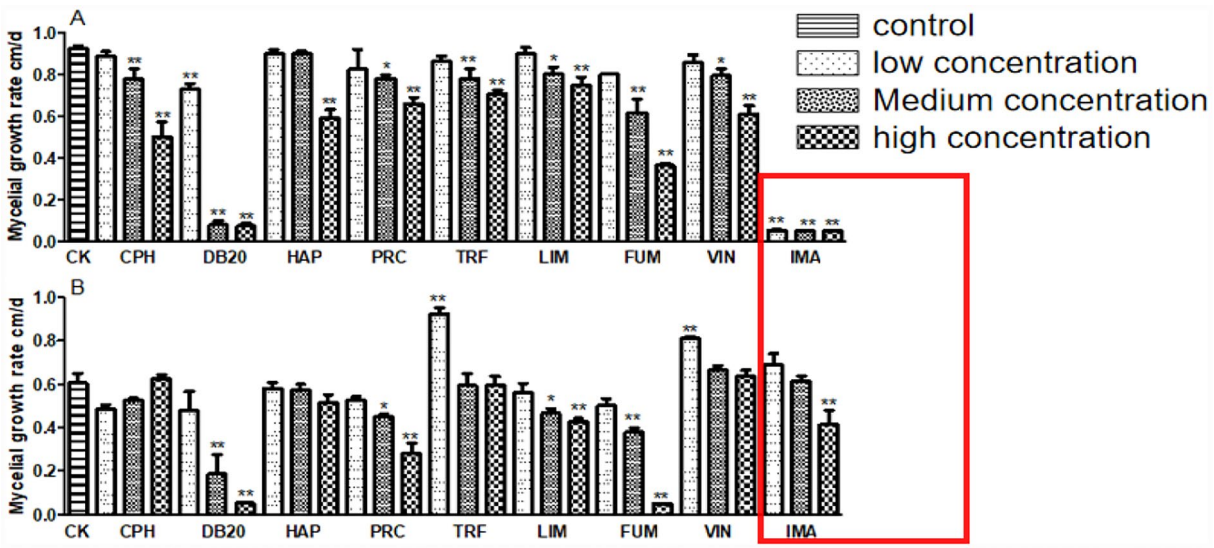



**Fig. 4** Indoor screening of prevention and control agents. **A** Effects of different agents on the growth of mycelia *X. ganodermophthora*; **B** effects of different agents on the growth of *G. lucidum*; Compared with CK, $^{*}P < 0.05$, $^{**}P < 0.01$

of 4–10 pH, and the optimum growth pH is 5–7 [13]. However, we found that the mycelia of pathogenic bacteria maintained rapid growth between pH 2–9, and that growth was fastest at pH 5. In terms of light, the fruit body of *G. lucidum* is sensitive to light and exhibits light-oriented growth [14]. Our data showed that the pathogen was also conducive to mycelial growth under 12 h of light. The above results suggest that it is not feasible to physically eliminate *X. ganodermophthora* according to biological characteristics.

#### Evaluation of specific inhibitors of pathogenic microorganisms in continuous cropping *G. lucidum*

The outcomes are presented in Fig. 4. All nine medications were discovered to have inhibitory effects on the growth of *X. ganodermophthora*. Notably, Acticide DB20 and Imazalil were found to strongly impede the development of *X. ganodermophthora* even at low concentrations. The suppressive impact of Imazalil was the most notable. The growth of *G. lucidum* was enhanced by a high concentration of Hachemical CPH ether, as well as low concentrations of Triforine, Vinclozolin, and Imazalil (Fig. 4A and B). This indicates that Triforine, Vinclozolin, and Imazalil have the potential to be used as agents to mitigate the challenges associated with continuous planting of *G. lucidum*. However, further research and verification are required to confirm these findings.

Following the plate experiment (Fig. 5) we found that the colonies of pathogenic bacteria in the CK group had a white color during the initial phase of growth. Three days later, the hyphae surrounding the inoculation block initiated the release of yellow pigments, and the conidia started to develop. After 6 days, the mycelium fully covered the plate. Nevertheless, when exposed to a low concentration of Imazalil the mycelium of the pathogen exhibited no growth for a duration of 7 days. Compared with the control group, the *G. lucidum* plate mycelium treated with a low concentration of Imazalil showed an improved growth rate and density. The plate experiment demonstrated that a low dose of Imazalil effectively suppressed the growth of the pathogen's mycelium, while simultaneously stimulating *G. lucidum* mycelium growth.

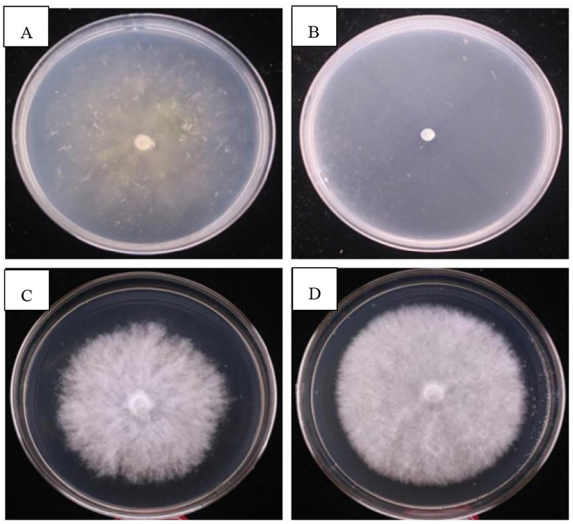



**Fig. 5** Effect of imazali on *X. ganodermophthora* and *G. lucidum* mycelium growth. **A** The growth of *X. ganodermophthora* in group CK. **B** Effects of low concentration of Imazalil on the growth of *X. ganodermophthora*. **C** Growth of *G. lucidum* in group CK. **D** The effect of low concentration of Imazalil on the growth of *G. lucidum*

#### Effect of Imazalil on fruiting bodies of *G. lucidum*

As shown in Fig. 6 and Table 3. The growth cycle of *G. lucidum* in the first tide is 35 days. The growth rate of *G. lucidum* in the tie-back test group showed a noticeable decrease, resulting in a prolonged growth cycle. The *G. lucidum* count in a single pack of the tie-back test group, low-dose experimental group, and medium-dose experimental group also increased while the width decreased. The low-dose control group showed a 0.44% increase in bag output compared to the blank control group, whereas the medium-dose control group showed a 1.14% increase compared to the blank control group. The tie-back test group exhibited a 6.96% decrease in the production of a single bag compared to the blank control group. Under the action of Imazalil, the low-dose experimental group and the medium-dose experimental group showed an increase of 1.92% and 2.09% in output, respectively, compared to the tie-back test group. The first tide of *G. lucidum* data demonstrated that Imazalil has a specific stimulating impact on the growth of *G. lucidum*, aligning with the findings of the plate experiment.

As shown in Fig. 7 and Table 3. The growth cycle of *G. lucidum* in the second tide is 45 days. The number of single bag of *G. lucidum* increased significantly in the tie-back test group, the low-dose experimental group, and the medium-dose experimental group. The number of single bag of *G. lucidum* in the blank control group, the low-dose control group, and the medium-dose control group also increased slightly. Correspondingly, the length and width of the second tide of *G. lucidum* in each group noticeably decreased. The low-dose control group showed an approximate 3.53% increase in yield per bag compared to the blank control group, while the high-dose control group showed a 24.92% increase. Conversely, the tie-back test group exhibited a 12% decrease in yield per bag compared to the blank control group. Under the influence of Imazalil,

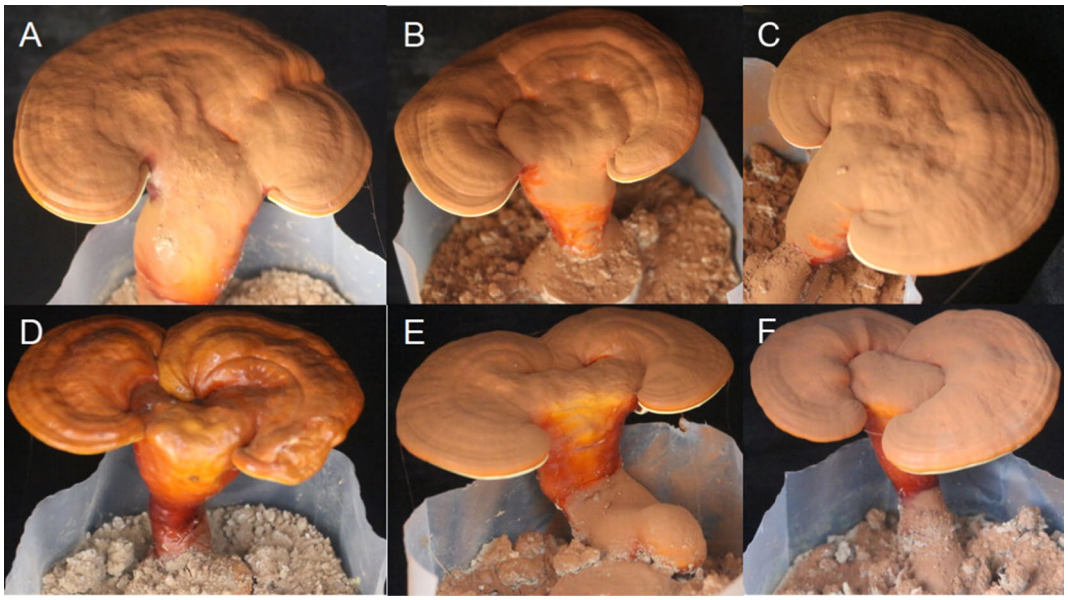



**Fig. 6** The growth situation of first tide. **A** Blank control group; **B** Low dose control group; **C** medium dose control group; **D** tie-back test group; **E** low dose experimental group; **F** medium dose experimental group; the picture below is the same

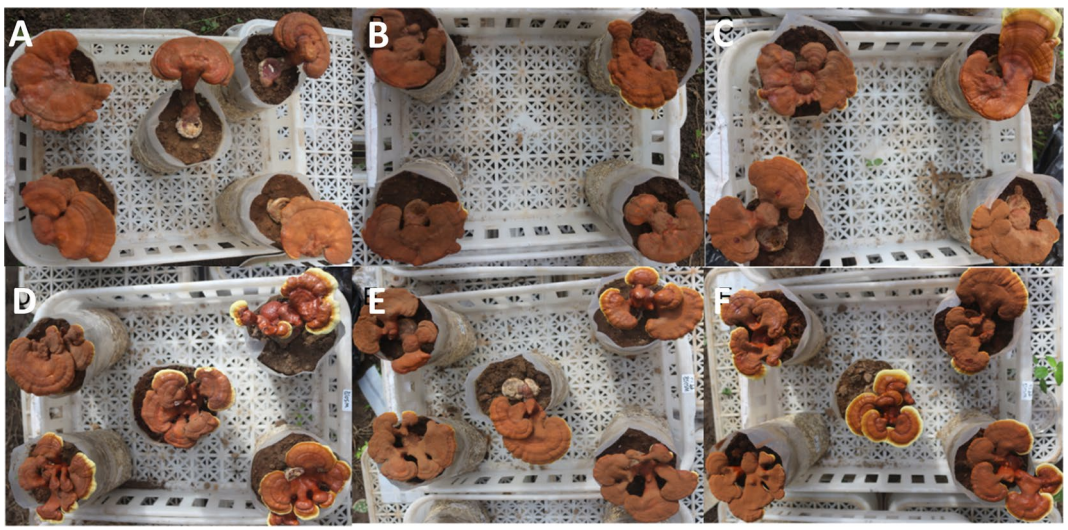



**Fig. 7** The growth situation of second tide. **A** blank control group; **B** low dose control group; **C** medium dose control group; **D** tie-back test group; **E** low dose experimental group; **F** medium dose experimental group; the picture below is the same

the low-dose and medium-dose experimental groups showed a respective increase in yield per bag of 28.80% and 47.65% compared to the reinoculation experimental group. The data from the second tide of *G. lucidum* demonstrates that Imazalil has a significant inhibitory effect on *X. ganodermophthoras* growth.

## Conclusions

Currently, the issue of continuous cropping obstacles in *G. lucidum* cultivation represents a primary constraint impeding the development of the *G. lucidum* industry [15, 16]. The persistent challenge of continuous farming in *G. lucidum* is commonly attributed to alterations in soil microbial features and the autotoxicity of *G. lucidum* products [17–19]. Ma et al. [20] think in continuous cropping soil microbial population, bacteria have

**Table 3** Agronomic traits of first tide and second tide

| Experimental Group | Agronomic traits of first tide | | | Agronomic traits of second tide | | |
|---|---|---|---|---|---|---|
| | Yield/g | Quantity/num. | Drying rate/% | Yield/g | Quantity/num. | Drying rate/% |
| Blank control group | 38.64 ± 2.13 | 1.30 ± 0.48 | 35.91% ± 0.67% | 28.33 ± 3.18 | 1.88 ± 0.83 | 61.72% ± 9.60% |
| Low dose control group | 38.81 ± 2.62 | 1.33 ± 0.50 | 37.79% ± 1.38% | 29.33 ± 3.88 | 2.00 ± 0.76 | 63.92% ± 8.76% |
| Medium dose control group | 39.08 ± 2.26 | 1.38 ± 0.52 | 38.10% ± 0.67% | 35.39 ± 4.79 | 2.20 ± 0.84 | 57.52% ± 6.35% |
| Tieback experimental group | 35.95 ± 3.80 | 1.56 ± 0.53 | 35.91% ± 0.67% | 24.93 ± 5.83 | 4.20 ± 0.84 | 60.81% ± 5.43% |
| Low dose experimental group | 36.64 ± 3.04 | 1.44 ± 0.73 | 35.91% ± 0.67% | 32.11 ± 3.51 | 3.75 ± 0.50 | 63.57% ± 3.51% |
| Medium dose experimental group | 36.70 ± 3.69 | 1.67 ± 0.50 | 35.91% ± 0.67% | 36.81 ± 4.18 | 4.00 ± 0.93 | 60.89% ± 4.49% |

strong inhibition on allelopathy of *G. lucidum* thallus. Among fungi, Trichoderma exhibited the most pronounced inhibitory impact on the growth of *G. lucidum* among the fungal microorganisms, with penicillium and Streptospora following suit. Further, Zhang et al. [21] believe that autotoxicity can impact the development of plant somatic cells, the permeability of cell membranes, enzyme activity, and the absorption and utilization of nutrients [21], thus affecting the growth of crops, resulting in continuous cropping obstacles. To break through this limitation, many researchers have explored the generation and prevention mechanisms of *G. lucidum* continuous cropping disorder to improve the issue of *G. lucidum* continuous cropping disorder. Ja et al. [22] have employed different bacteriostatic agents to treat *G. lucidum* and hinder the growth of harmful bacterial hyphae. Wu et al. [23] fumigated continuous cropping soil with liquid ammunition. Yuan et al. [24] treated continuous cropping soil with lime soaking and uniform sprinkling. All of the above can improve the continuous cropping obstacle of *G. lucidum*.

Imazalil, a compound that inhibits the production of sterols, was granted approval for use in 1979 [25–27]. The primary mechanism by which it exhibits its antibacterial properties is through the inhibition of ergosterol production. It is mostly employed as a bacteriostatic agent or for fruit preservation [28–30]. Imazalil has high sensitivity and good control effect on pathogenic bacteria such as wet blister disease and crown rot [31–33]. There have been many studies on the effects of *G. lucidum* continuous cropping obstacles on soil microorganisms, one of which is *X. ganodermophthora*. Hence, this investigation uses Imazalil as a means to inhibit and manage *X. ganodermophthora*. These findings demonstrated that Imazalil has a substantial inhibitory impact on the *X. ganodermophthora* growth.

The specific primers used to amplify the soil samples showed that the DNA from the CK group and GL0 group did not produce any amplified bands, however the DNA from the GL2 group and GL4 group successfully produced amplified bands. Simultaneously, the findings of the flat plate confrontation test demonstrated a notable inhibitory impact of *X. ganodermophthora*, a decomposing fungus of *G. lucidum*, on the growth of *G. lucidum* hyphae. Based on the aforementioned investigations, we concluded that *X. ganodermophthora*, a microorganism that causes spoiling in *G. lucidum*, is one of the pathogenic bacteria that hinders the ongoing cultivation of *G. lucidum*. Through the examination of the biological attributes of pathogenic bacteria, our aim was to investigate potential physical methods for preventing and managing these germs, as well as identifying specific inhibitors to effectively achieve the goal of prevention and control. This pathogen's hyphae exhibit vigorous development within a temperature range of 25–32 °C. High temperature, low temperature, total darkness and total light could h not effective for the growth of *X. ganodermophthora* hyphae. The hyphae become inactive at a temperature of 60 °C or at pH 2. Indoor control agent screening revealed that even at low concentrations, Imazalil can effectively hinder the growth of *X. ganodermophthora* and stimulate the growth of *G. lucidum*. Here, we tested the effectiveness of imazalil in controlling continuous crop growth through field experiments.

In the early stage of laboratory, ITS1 amplicon sequencing was carried out on adjacent wild soil, 1-year, 2-year and 4-year cultivated *G. lucidum* soil by Illumina MiSeq platform. The results revealed a decrease in fungal diversity in the *G. lucidum* cover soil as the number of consecutive cropping years increased. The covering soil of *G. lucidum* planted for four years only contained Basidiomycetes, Ascomycetes and a small amount of Mortierella. The relative abundance of fungi in soil changed greatly with the continuous cropping years, among which Basidiomycota increased significantly, Ascomycota decreased significantly, but Mortierella had no significant difference. In the previous experiment, 150 $m^2$ of soil planted for four years were treated with 45 kg of lime. The disease rate of *G. lucidum* can be reduced by about 28.28%,

significantly improving *G. lucidum* diseases. At the same time, the lime soaking treatment of *Ganoderma lucidum* continuous cropping soil changed the fungal structure in the covering soil, which was mainly manifested in the decrease in the relative abundance of Ascomycota and the increase in the relative abundance of Basidiomycota [24, 34]. This investigation established that the utilization of Imazalil effectively suppressed the proliferation of X. ganodermophthora spoiling in *G. lucidum*. Therefore, we hypothesize that Imazalil along with lime may be a promising strategy to overcome continuous cropping obstacles in *G. lucidum* and to promote the development of the *G. lucidum* industry.

**Abbreviations**

ANOVA Analysis of variance
PDA Potato dextrose agar

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Not applicable.

**Author contributions**
JF, QH, and FC designed the experiments. QH, QL, and XL performed the experiments. JF, HS, JZ and XW contributed reagents and materials. JF, QH and FC drafted the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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