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Research Article

Identification of Pathogens and Laboratory Activity Test of Kiwifruit Rot Disease in Guizhou Province, China

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Kiwifruit (*Actinidia* spp.) postharvest decay is common in China, which can cause serious economic losses to kiwifruit industry. In order to further clarify the pathogen of kiwifruit rot disease in Guizhou Province, the rotten fruits of kiwifruit (cultivar "Jinyan") were collected, and the pathogenic fungi were identified by isolation and purification, pathogenicity test, morphological characteristics, and analysis of rDNA-ITS sequences. The results showed that the pathogenic fungi of kiwifruit rot disease were *Diaporthe phaseolorum* and *Fusarium tricinctum*. Meanwhile, the results showed that all the tested agents had a certain inhibitory effect on *Diaporthe phaseolorum* and *Fusarium tricinctum*. Among them, 33.5% quinolone SC had the best inhibitory effect on *Diaporthe phaseolorum* with an EC_{50} value of 9.67 mg/L, and 25% fludioxonil SC had the best inhibitory effect on *Fusarium tridentatus* with the EC_{50} value of 13.13 mg/L. The results will provide a reference for the control of kiwifruit rot disease.

1. Introduction

Kiwifruit (*Actinidia* spp.) has soft meat, sour and sweet taste, rich in vitamin C, sugars, and a variety of essential amino acids for human body. It has a high nutritional and economic value and is deeply loved by consumers, which is known as "super fruit" and "king of fruit." Kiwifruit is native to China, and more than 30 countries have engaged in large scale and industrialized artificial cultivation of kiwifruit industry. In 2020, the planting area of kiwifruit in China was about 193000 hectares and the output was about 2.291 million tons, accounting for more than 68% and 50% of the world, respectively [1]. Guizhou Province is located in the west of China, and its geographical and climatic conditions are suitable for the growth of kiwifruit and have been one of the main kiwifruit planting areas in China. By 2020, the cultivated area has reached 4.51×10^4 hm^2 [2].

In recently years, with the rapid expansion of the kiwifruit planting area, the problem of rot disease has become increasingly prominent [3–5]. At present, postharvest rot disease of kiwifruit occurs widely around the world, causing serious economic losses during fruit storage, transportation, and sales [6, 7]. Kiwifruit rot disease mainly occurs in the postharvest period and storage stage of the fruit. Its main symptoms are the formation of round or oval brown lesions on the peel, a water-stained ring on the edge of the lesion, and the color of the flesh of the lesion. It is milky white, and the pulp at the junction between disease and health is water-stained, often forming perforated rot. In severe cases, the whole fruit rots completely [8, 9]. The pathogenic microorganism of kiwifruit rot is rich in diversity. At present, the pathogenic microorganism that have been reported are mainly *Botryosphaeria dothidea* [10, 11], *Phomopsis* spp. [12, 13], and *Pestalotiopsis* spp. [7, 10]. *Alternaria alternata*, *Plectosphaerella cucumerina*, *Neofusicoccum parvum*, *Phomopsis* spp., and *Fusarium oxysporum* have been reported as pathogens of kiwifruit rot in Guizhou Province, China [10, 14–17]. However, research studies on rot disease of Guizhou kiwifruit are basically concentrated on "Guichang" kiwifruit and "Hongyang" kiwifruit varieties, and there are few reports on Guizhou "Jinyan" kiwifruit varieties.

2. Materials and Methods

2.1. Isolation and Purification of the Pathogens. The rotten kiwifruit was collected from Gubao town (106.525230°E,

26.852491°N), Maijia town (106.626288°E, 26.711823°N), and Machang town (106.223953°E, 26.447156°N) in Guizhou Province, China. A total of 290 samples were collected, packaged in a clean ziplock bag, and then taken back to the laboratory store in a 4°C refrigerator for pathogen isolation. The kiwifruit is first rinsed with tap water and then dried. The infected tissues (0.5 × 0.5 cm size) were soaked in 75% alcohol for about 30 s, rinsed with sterile water 3 s, and then plated the tissues on the PDA plates. After that, the PDA plates were maintained in a constant temperature incubator at 26°C without light. After culturing for 3 days, all the strains were cultured three times on the new PDA plates using a single spore technique to ensure purity. Finally, the purified strains were stored at 4°C for further use.

2.2. Pathogenicity Test. Pathogenicity tests were performed by inoculating the fungus on the puncture site of the surface of healthy and nearly mature kiwifruits, and the kiwifruits were incubated in an incubator in a 26°C constant temperature incubator with a humidity of 60% and a photoperiod of 14 L : 10 D. The surface of healthy and nearly mature kiwifruits inoculated with sterile water served as a control. After 12 days of inoculation, some symptoms have been observed on the surface. The causal fungus in the infected kiwifruit surface was reisolated on the PDA plate as described above. The characteristics of the reisolated fungus were used to compare with its original culture.

2.3. Morphological and Molecular Identification. Individual colony was inoculated on the PDA plate and maintained in a constant temperature incubator at 26°C without light for 8 days. Then, the morphology was identified by both eyes and an inverted microscopy (ECLIPSE Ni-E, Nikon Corporation, Japan). The total DNA of the tested strain was extracted with the Ezup column fungal genomic DNA extraction kit (B518259-0050, Sangon Corporation Shanghai, China), and the rDNA-ITS sequence was amplified by primers ITS1 (5′-TCCGTAGGTGAACCTG CGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The total reaction volume is 25 μL: 12.5 μL 2xEs Taq Mix, 1 μL each primer, 1 μL DNA, and 9.5 μL ddH_2O. The polymerase chain reaction (PCR) reaction conditions were predenaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 56°C for 1 min, 72°C for 1 min, and final extension at 72°C for 7 min. After amplification, the PCR product was sequenced at Sangon Corporation (Shanghai, China) and searched for sequence similarity with the National Center of Biotechnology Information (NCBI) database.

2.4. In Vitro Antifungal Activity Test. The in vitro antifungal activities of 11 kinds of fungicides, 33.5% quinolone SC (Shanghai Hulian biopharmaceutical Co., Ltd., China), 250 g/L propiconazole EC (Shandong Xinxing pesticide Co., Ltd., China), 25% myclobutanil EC (Zhejiang Yifan Biotechnology Group Co., Ltd., China), 25% fludioxonil SC (Jiangsu Syngenta Nantong crop protection Co., Ltd., China), 0.3% eugenol AP (Jiangsu Nantong Shenyu green Pharmaceutical Co., Ltd., China), 80% ethylicin EC (Henan Kebang Chemical Co., Ltd., China), 100 g/L cyazofamid SC (Henan Guangnong pesticide factory, China), 1% Osthol AP (Inner Mongolia Qingyuanbao Biological Technology Co., Ltd., China) 25% cupric-ammonium complexion (Henan Anyang Guofeng Pesticide Co., Ltd., China), 430 g/L tebuconazole SC (Jiangsu Renxin Crop Protection Technology Co., Ltd., China), and 80% zineb WP (Shandong Xinxing Pesticide Co., Ltd., China) and 5 kinds of essential oils (patchouli essential oil (Guangzhou Biotechnology Co., Ltd., China), fennel essential oil (Beijing Maosi Trading Co., Ltd.), garlic essential oil (Beijing Maosi Trading Co., Ltd.), clove essential oil (Beijing Maosi Trading Co., Ltd.), and benzoin essence oil (Beijing Maosi Trading Co., Ltd.) were tested [18]. The inhibition rates I (%) are calculated by the following formula, where C (cm) and T (cm) represent the fungi diameters of the CK and treated PDA plates, respectively. Meanwhile, the EC_{50} values of 11 kinds of fungicides and 5 kinds of plant essential oils against *Diaporthe phaseolorum* and *Fusarium tricinctum* were calculated with the SPSS 19.0 software.

$$\text{Inhibition rate, } I\ (\%) = \frac{(C - T)}{(C - 0.4)} \times 100. \quad (1)$$

3. Result

3.1. Morphological Identification. The hyphae of strain F1 are fluffy and white in the early stage of growth. The center of the hyphae appears yellowish-brown, and the edges are white on the 3rd day. On the 8th day, the diameter of the colony is overgrown in the Petri dish. The hypha on the front is dark gray (Figure 1(a)), the back of the PDA medium is dark brown (Figure 1(b)), and the hyphae are transparent with many branches and segments (Figure 1(c)).

The hyphae of strain F2 are fluffy, appearing white at the initial stage of growth, with irregular edges and slow growth. On the 3rd day, the mycelium produces pink pigment. Then, on the 10th day, the diameter of the colony grows over the Petri dish, and the color of the bottom of the medium gradually changes to rose red on the front (Figure 1(d)), with yellow on the back (Figure 1(e)) and many hyphae branches (Figure 1(f)).

3.2. ITS Sequence Identification. The sequences of F1 and F2 strains were uploaded to NCBI to obtain the GenBank numbers of ON566024 and ON566025. Phylogenetic trees of the ITS sequence were constructed based on the N-J and M-L methods, as shown in Figure 2, and the strain F1 was classified as *Diaporthe phaseolorum* with the similarity of 99% and 85%, respectively. Meanwhile, Figure 3 shows that the strain F2 was classified as *Fusarium tricinctum* with bootstrap values of both 100% ((N-J method) and (M-L method)).

3.3. Pathogenicity Determination. Pathogenicity was determined by the stab inoculation method, and the results are shown in Figure 4. Figure 4 shows that the *Diaporthe phaseolorum* (strain F1) and *Fusarium tricinctum* (strain F2) can cause kiwifruit rot. Among them, the diameter of kiwifruit rot disease caused by F1 was 3.93 cm (Figure 4(b)),

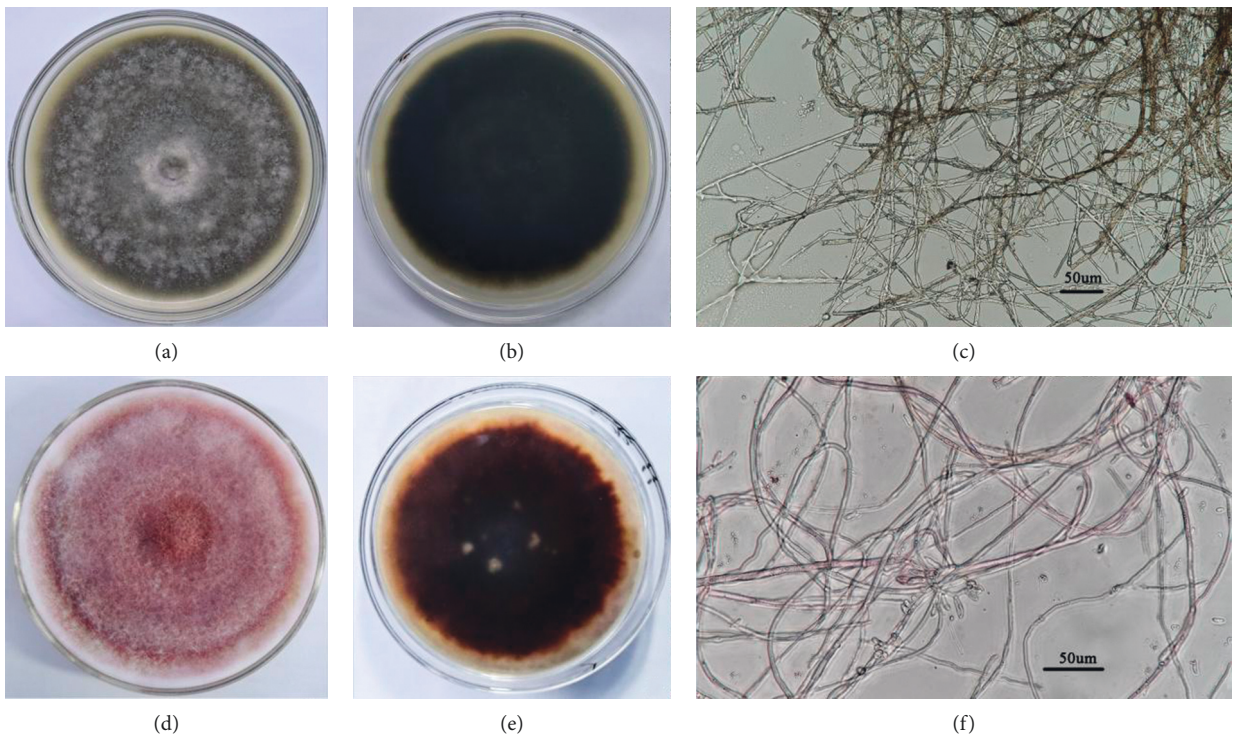

Figure 1: Morphological characteristics of F1 and F2 strains. (a)–(c) The front, back, and hyphal morphology of F1 colonies, respectively; (d)–(f) The front, back, and hyphal morphology of F2 colonies, respectively.

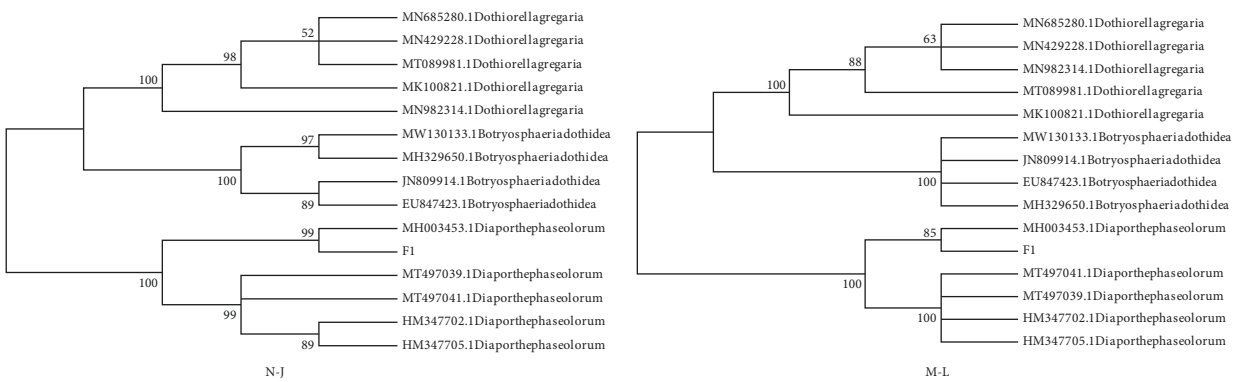

Figure 2: Phylogenetic trees of the ITS sequence of strain F1 constructed based on the N-J and M-L methods.

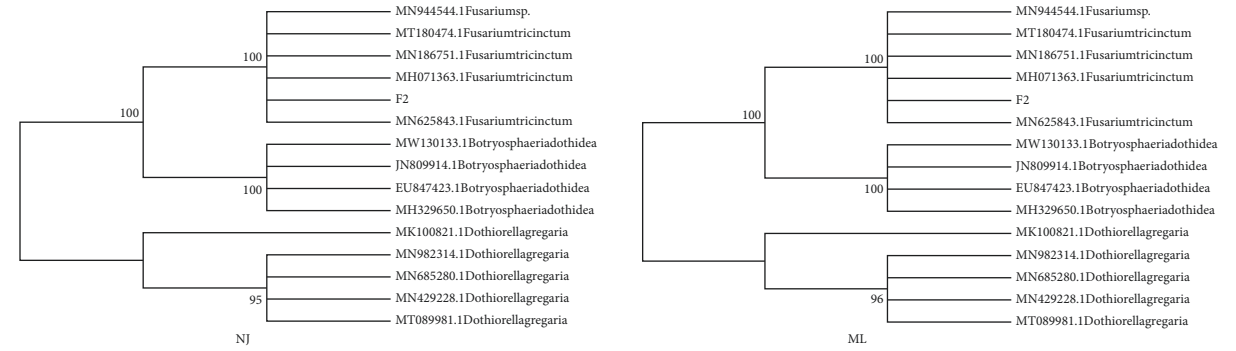

Figure 3: Phylogenetic trees of the ITS sequence of strain F2 constructed based on the N-J and M-L methods.

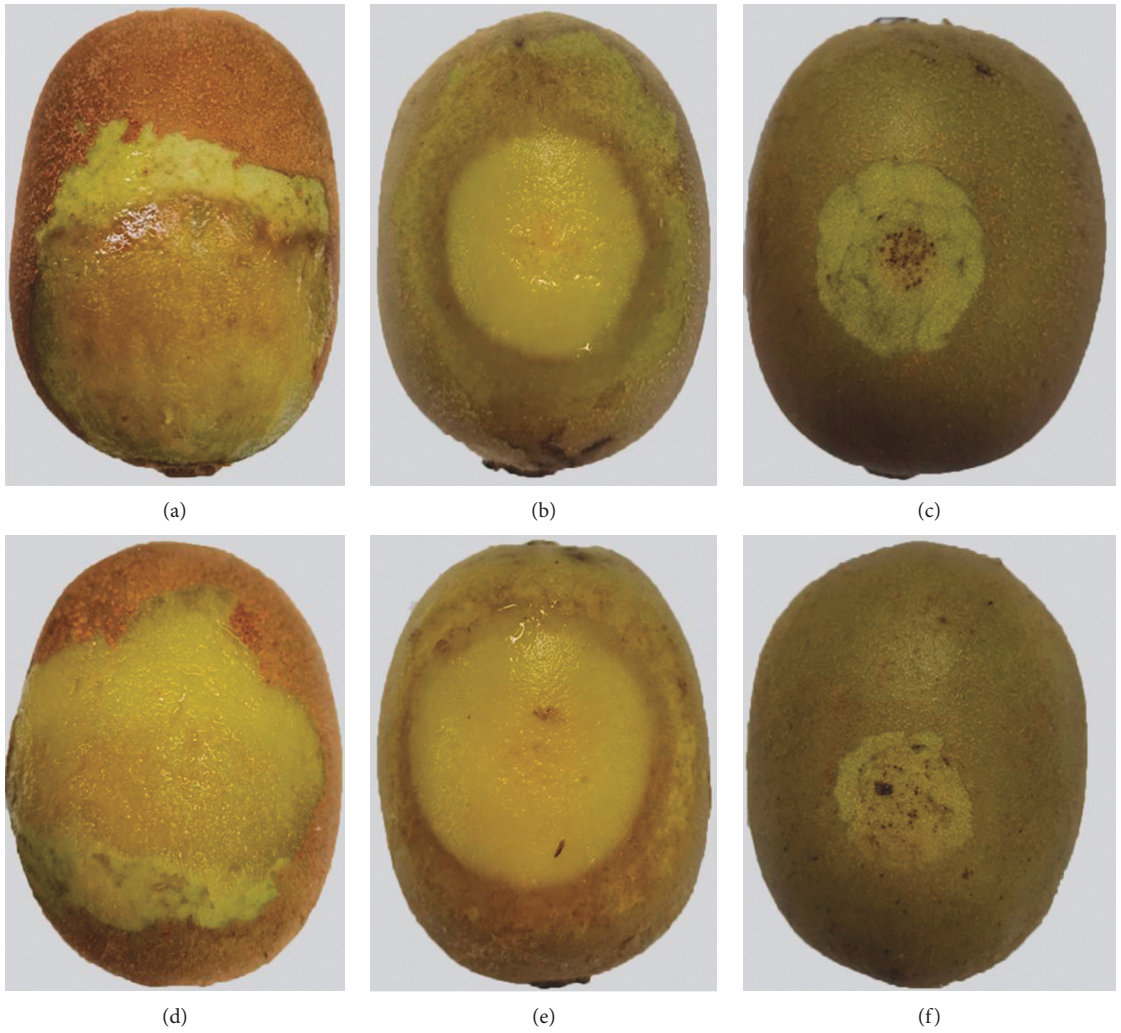

Figure 4: The symptoms of F1 and F2 strains in the pathogenicity test. (a)-(b) The pathogenicity test by F1 strain. (c) CK group for strain F1. (d)-(e) Pathogenicity test by F2 strain. (f) CK group for strain F2.

and F2 caused kiwifruit rot disease with a diameter of 4.25 cm (Figure 4(e)). The diseased kiwifruit was reisolated, and the strains with the same morphological characteristics as the original inoculated strains were obtained, which met the requirements of Koch's law.

3.4. In Vitro Antifungal Activity. It can be seen from Table 1 that the test fungicides and plant essential oils revealed different degrees of inhibition on the growth of *Diaporthe phaseolorum* and *Fusarium tricinctum*. Especially, 33.5% quinolone SC showed the best inhibitory effect against *Diaporthe phaseolorum* with the EC_{50} value of 9.67 mg/L; meanwhile, 25% fludioxonil SC had an EC_{50} value of 13.13 mg/L against *Fusarium tricinctum*, which were even better than those of other fungicides and plant essential oils.

4. Discussion

Postharvest rot disease of kiwifruit occurs globally, which has a significant impact on the quality and flavor of kiwifruit.

Table 1: The EC_{50} values of the test fungicides and plant essential oils against *Diaporthe phaseolorum* and *Fusarium tricinctum*.

Treatment	EC_{50} (mg/L)	
	Diaporthe phaseolorum	*Fusarium tricinctum*
33.5% quinolone SC	9.67	34.53
250 g/L propiconazole EC	12.55	59.71
25% myclobutanil EC	16.54	13.66
25% fludioxonil SC	18.54	13.13
0.3% eugenol AP	48.81	111.32
80% ethylicin EC	85.10	82.55
100 g/L cyzzofamid SC	91.66	30.83
430 g/L tebuconazole SC	103.78	58.89
80% zineb	106.27	642.17
1% osthol AP	215.36	15.52
25% cupric-ammonium complexion	430.73	49.04
Patchouli essential oil	115.00	122.89
Fennel essential oil	131.48	109.57
Garlic essential oil	156.11	101.74
Clove essential oil	163.87	132.96
Benzoin essence oil	163.96	136.67

At present, it has caused significant economic losses to the kiwifruit industry. Therefore, the identification of pathogens of kiwifruit postharvest rot disease is of great significance for industrial development. In this study, 2 pathogens classified as *Diaporthe phaseolorum* and *Fusarium tricinctum* were obtained from the rotten fruits of kiwifruit (cultivar "Jinyan") which were collected from Guizhou Province, China. *Diaporthe phaseolorum* has a higher separation rate, and the asexual form of the fungus is *Phomopsis* spp. [19]. *Phomopsis* spp. has also been reported many times in other varieties in Guizhou Province, for example, "Guichang" kiwifruit [12, 20] and "Hongyang" kiwifruit [21]. Our results showed that the main pathogen of kiwifruit (cultivar "Jinyan") rot disease in Guizhou Province was related to other strains. In addition, *Phomopsis* spp. was an important pathogen of kiwifruit rot disease in other regions, such as "Xuxiang" kiwifruit in Hubei and Shaanxi [22], "Jinyu" kiwifruit in Hubei, and "Golden" kiwifruit in Wuhan. The "Jinmei" kiwifruit [23] detected *Phomopsis* spp. as an important pathogen. It indicated that *Phomopsis* spp. was an important pathogen of kiwifruit rot disease among different strains and regions, and it caused harm to various strains of kiwifruit in the whole country.

Fusarium tricinctum is the first pathogenic fungus found in the identification of kiwifruit rot disease pathogens in Guizhou Province. This pathogen has not been found to infect kiwifruit in previous studies. *Fusarium* is widely distributed in nature and is one of the most important phytopathogens discovered so far [24]. It can cause a variety of plants and their fruits to rot. Among them, *Fusarium tricinctum* can cause apple moldy heart disease [25], garlic root rot [26], lily *Fusarium* wilt [27], and potato dry rot [28]. Yang et al. [29] found that there were significant differences in the pathogenicity and severity of *Fusarium* in different provinces and between different places in the same province. Because *Fusarium tricinctum* are more harmful to fruits and fruit trees and have a wide range of damage; they should be paid attention to in field prevention and control.

In recent years, the prevention and treatment of kiwifruit fruit rot disease gradually attracted the attention of the world kiwifruit industry. In this study, the in vitro inhibitory effects of 16 kinds of fungicides and plant essential oils against *Diaporthe phaseolorum* and *Fusarium tricinctum* were determined. The results showed that all the tested agents had a certain inhibitory effect on the four pathogenic fungi. Among them, 33.5% quinolone SC had the best inhibitory effect on *Diaporthe phaseolorum* and 25% fludioxonil SC had the best inhibitory effect on *Fusarium tricinctum*. At present, there are many screening studies on the prevention and control of kiwifruit rot disease. The commonly used agents are mainly carbendazim, tebuconazole, prohydan, flusilazole, benomyl, and thiophanate-methyl. Previous studies have shown that ethylicin could significantly inhibit the mycelial growth of *P. macrospore*, *Botryotinia fuckeliana*, *Botryosphaeria dothidea*, and *Fusarium proliferatum* [30]. 42.4% azole ether-fluranil SC, 40% flusilazole EC, and other three kinds of fungicides had a significant inhibitory effect on *Pestalotiopsis gracilis* [31]. Curcumin has a significant inhibitory effect on the growth of *Diaporthe phaseolorum* mycelium [32]. But serious pesticide residue problems will come with the long-term use of pesticides [33], for example, pesticide residues are the main bottleneck for my country's kiwifruit export volume and price increase [34]. Therefore, there is an urgent need for research and development to find alternatives to pesticides [35]. To sum up, the optimal control agents for various pathogens are different; this may be related to kiwifruit varieties and local geographic climate. Based on the results of the abovementioned in vitro screening of fungicides against kiwifruit rot pathogens, the field control effect tests of 33.5% quinolone SC and 25% fludioxonil SC against kiwifruit rot disease can be carried out in our next work, so as to provide an effective prevention and control method of kiwifruit rot disease in Guizhou Province.

5. Conclusion

In conclusion, our results showed that *Diaporthe phaseolorum* and *Fusarium tricinctum* were the pathogenic fungi of kiwifruit (cultivar "Jinyan") rot disease in Guizhou Province. Meanwhile, 33.5% quinolone SC had the best inhibitory effect on *Diaporthe phaseolorum* and 25% fludioxonil SC had the best inhibitory effect on *Fusarium tricinctum*. Our study could provide a theoretical basis for the effective control method of kiwifruit rot disease in Guizhou Province.

Data Availability

The data used to support this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Tao Wang and Yanling Ren contributed equally to this work.

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