Mathematical Analysis of the Inhibitory Effects of Ten Kinds of Fungicides Applied on Apple

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Abstract. Apple ring rot is one of the three major diseases of apple, which seriously affects the yield and quality of apple. At present, chemical agents are mainly used to prevent and control apple ring rot. In this study, 10 kinds of low virulence fungicides were selected. The inhibitory effects of 10 kinds of fungicides on Botryosphaeria dothidea were determined. The results indicated that 50% carbendazim and 70% thiophanate methyl had the best mycelial growth inhibition effect among 10 kinds of fungicides. The inhibitory effect of 50% carbendazim on hyphae was 91.63% under the concentration of 400 times dilution, the inhibitory effect of 70% thiophanate methyl on hyphae was 90.31% under the concentration of 500 times dilution, and there were recommended to use.

Keywords: Apple Ring Rot, Mycelial Growth Inhibition Effect, Fungicide.

1. Introduction
Apple is a kind of fruit with high nutritional value, which is loved by people all over the world [1]. China is the world's largest apple production country, but the output per unit area is not high, the main reason is the serious occurrence of diseases [2]. Disease has a great influence on the yield and quality of apple, among which apple ring rot, apple canker and apple spot leaf drop are the three major diseases [3]. Apple ring rot can not only affect apple trees, it can also affect pears, apricots, peaches, chestnuts and other fruit trees [4]. Due to the heavy losses caused by apple ring rot, agricultural control and biological control methods can't quickly and effectively achieve the ideal control purpose. At present, chemical control is still the main method in production [5].

Therefore, in this study, 10 kinds of fungicides with low toxicity and low residue, which are commonly used to prevent and control apple ring rot, were selected as the test fungicides. In the effective dilution times range of fungicides, the inhibitory effect on apple ring rot was studied to provide some scientific basis for the prevention and control of apple ring rot in agriculture.
2. Materials and methods

2.1. Test strains
Botryosphaeria dothidea was the test strain, which was extracted, isolated and purified from the apple ring-line disease strain. The pathogenic strain was accurately identified and preserved in the laboratory.

2.2. Test fungicides
In this study, 10 kinds of fungicides were selected as test fungicides. According to the effective dilution times range of each fungicide, 5 dilution multiples were set, as shown in Table 1.

| Fungicide Formulations       | Dilution times 1 | Dilution times 2 | Dilution times 3 | Dilution times 4 | Dilution times 5 |
|-----------------------------|------------------|------------------|------------------|------------------|-----------------|
| 3% zhongshengmycin WP        | 100              | 200              | 400              | 800              | 1000            |
| 12.5% Diniconazole WP        | 2000             | 2100             | 2200             | 2300             | 2400            |
| 250g/L Azoxystrobin SC       | 700              | 900              | 1100             | 1300             | 1500            |
| 430g/L Tebuconazole SC       | 3000             | 3250             | 3500             | 3750             | 4000            |
| 75% Chlorothalonil WP        | 600              | 650              | 700              | 750              | 800             |
| 25% Flusilazole WG           | 800              | 900              | 1000             | 1100             | 1200            |
| 80% Mancozeb WP              | 500              | 575              | 650              | 725              | 800             |
| 50% Carbendazim WP           | 400              | 700              | 1000             | 1300             | 1600            |
| 10% Difenoconazole WG        | 1000             | 1500             | 2000             | 2500             | 3000            |
| 70% Thiophanate methyl WP    | 500              | 800              | 1200             | 1600             | 2000            |

2.3. Production of medium
Preparation of PDA medium: peeled potatoes, cut them into pieces, weighed 200g, put them into 1000mL distilled water, and boiled them until they were soft and rotten. After the filtration residue, 20g glucose and 20g AGAR were added and stirred until melted. Distilled water was constant volume to 1000 mL, then divided into triangular flask for autoclave sterilization and set aside [6].

Preparation of media containing fungicides: the fungicide was prepared according to the dilution times set in Table 1. When the PDA medium was melted and cooled to about 60°C, the fungicide and PDA medium were prepared according to the ratio of 1:9 [7]. After fully shaking, the medium plate containing fungicide was prepared, and the PDA plate with equal volume of sterile water was taken as the control (CK) [8].

2.4. Mycelial growth rate method
The inhibitory effect of ten fungicides on the pathogen was tested by using growth rate method. The fungus cake was made on the outer edge of the fungal colony caused by apple ring streak with a 6 mm fungus punch [9]. The fungus cake was picked out with sterilized inoculation needle, and the mycelium side on the cake was placed in the center of the medium containing fungicides. The PDA medium without fungicides was used as the control group (CK) [10]. Each treatment was repeated three times in an incubator at 25°C for 6 days. The diameter of the colony was measured by vertical cross method and the inhibition rate of mycelium growth was calculated. The formula of mycelial growth inhibition rate is as follows:

Inhibitory rate = (control colony diameter - treatment colony diameter)/ (control colony diameter -6)
×100%  (1)

SPSS software was used to analyze the data.

3. Results and analysis

3.1. Inhibitory effect of 10 kinds of fungicides on Botryosphaeria dothidea
The inhibitory effects of 10 kinds of fungicides on Botryosphaeria dothidea were shown in Table 2. The results indicated that 10 fungicides had significant effect against Botryosphaeria dothidea. Among the 10 fungicides, the best inhibitory effect on the pathogen was 91.63% under the concentration of 10% difenoconazole 1000 times dilution, 50% carbendazim 400 times dilution and 80% mancozeb 500 times dilution. Secondly, the inhibitory effect on the pathogen was 90.31% under the concentration of 70% thiophanate methyl 500 times dilution and 10% difenoconazole 1500 times dilution. The inhibitory effects of other fungicides at different dilution ratios were all lower than 90%. The inhibition rate of 3% zhongshengmycin was all lower than 15.00% at the five concentrations, and the inhibitory effect was the worst, the results showed that 3% zhongshengmycin had little inhibitory effect on B. dothidea. 250g/L azoxystrobin and 430g/L tebuconazole were poor in inhibiting B. dothidea, among the five dilution times set, the inhibition rate was all lower than 50.00%.

Table. 2 Inhibitory effect of 10 fungicides against mycelial growth of B. dothidea

| Fungicide          | Inhibition rate (%) | Dilution times 1 | Dilution times 2 | Dilution times 3 | Dilution times 4 | Dilution times 5 |
|--------------------|---------------------|------------------|------------------|------------------|------------------|------------------|
| 3% zhongshengmycin | 14.31 a             | 12.38 a          | 7.84 b           | 5.09 c           | 3.30 c           |
| 12.5% Diniconazole | 75.65 a             | 64.24 b          | 56.95 c          | 49.11 d          | 22.01 e          |
| 250g/L Azoxystrobin| 44.98 a             | 36.27 b          | 33.52 bc         | 29.39 c          | 23.43 d          |
| 430g/L Tebuconazole| 39.48 a             | 28.02 b          | 23.89 bc         | 21.14 c          | 7.84 d           |
| 75% Chlorothalonil | 61.69 a             | 54.20 b          | 48.04 c          | 39.23 d          | 36.15 d          |
| 25% Flusilazole    | 66.09 a             | 49.80 b          | 44.08 c          | 33.07 d          | 18.54 e          |
| 80% Mancozeb       | 91.63 a             | 77.10 b          | 48.48 c          | 15.90 d          | 3.13 e           |
| 50% Carbendazim    | 91.63 a             | 89.43 b          | 85.03 c          | 81.07 d          | 70.06 e          |
| 10% Difenoconazole | 91.63 a             | 90.31 ab         | 83.27 b          | 68.74 c          | 47.16 d          |
| 70% Thiophanate-methyl | 90.31 a         | 88.99 ab         | 87.23 b          | 76.66 c          | 72.70 d          |

Note: Values with different lowercase letters indicate significant difference (P<0.05), the same as below.

3.2. Comparison of inhibition effect of difenoconazole, carbendazim and mancozeb
The inhibitory effects of three fungicides, 10% difenoconazole, 50% carbendazim and 80% mancozeb, on B. dothidea were shown in Figure 1 and Table 2.
The results showed that the inhibition rate of the three fungicides was consistent at the lowest dilution ratio, and the inhibition effect was the best. In the case of high dilution times, 80% mancozeb had the worst inhibition effect, with the inhibitory rate of only 3.13%, followed by the inhibitory rate of 10% difenoconazole (47.16%), and the inhibitory rate of 50% carbendazim (70.06%).

With the increase of dilution ratio, only 50% carbendazim of the three fungicides showed good inhibition effect. In addition, the inhibition rate was much higher than that of 3% zhongshengmycin, 250g/L azoxystrobin, 430g/L tebuconazole, 75% chlorothalonil and 25% flusilazole at the lowest dilution ratio. In general, 50% carbendazim showed good inhibitory effect on \textit{B. dothidea} at the five dilution times set. The inhibition effect of 10% difenoconazole and 80% mancozeb was better when the dilution times was lower, but the inhibition effect was not ideal when the dilution times was higher.

3.3. Comparison of inhibition effect of carbendazim and thiophanate methyl

The inhibitory effect of 50% carbendazim and 70% thiophanate methyl was shown in Figure 2 and Table 2. Among the 10 kinds of fungicides, 50% carbendazim and 70% thiophanate methyl showed similar inhibitory effects on \textit{B. dothidea}, and both showed good inhibitory effects.

The inhibitory effect of 50% carbendazim on hyphae was 91.63% under the concentration of 400 times dilution, so it is recommended to use 400 times diluted concentration. When the concentration of 1600 times dilution, the inhibition effect on \textit{B. dothidea} was the worst, the inhibition rate was 70.06%.

The inhibitory effect of 70% Thiophanate methyl on hyphae was 90.31% under the concentration of 500 times dilution, and 88.99% under the concentration of 800 times dilution, there were no significant difference between them. When the concentration of 2000 times dilution, the inhibition effect on \textit{B. dothidea} was the worst, the inhibition rate was 72.70%.

Even at the highest dilution times, the inhibition effect is still higher than 70%, which is much higher than other fungicides. It can be concluded that 50% carbendazim and 70% thiophanate methyl have good overall inhibitory effect on \textit{B. dothidea}, 50% carbendazim and 70% thiophanate methyl are recommended to be used in the prevention and treatment of apple ring rot.
Figure 2. Inhibitory effect of 50% carbendazim and 70% thiophanate methyl against mycelial growth of B. dothidea

4. Conclusion
Ten kinds of fungicides had certain inhibitory effects on B. dothidea. In general, 50% carbendazim and 70% thiophanate methyl showed good inhibitory effect at different dilution times of the ten fungicides, so they were recommended to be used. At a low dilution times, 10% difenoconazole and 80% mancozeb showed good inhibitory effect, so the use of low dilution times was recommended. However, three fungicides, 3% zhongshengmycin, 250g/L azoxystrobin and 430g/L tebuconazole, have poor inhibitory effect and were not recommended to be used. Due to the lack of field proof in the laboratory, it is suggested to carry out further field experiment.

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