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Inhibitory effect of 14 fungicides on Pathogen of Coffee Leaf Blight *Phomopsis Heveicola*

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Research

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FAN Yan-ni[1], WU Wei-huai[2], HUANG Xin[2], HE Chun-ping[2], LIANG Yan-qiong[2], YI Ke-xian [2]

1.School of plant protection, Hainan university. Haikou, 571101, China
2.Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences/Key Laboratory for Monitoring and Control of Tropical Agricultural and Forest Invasive Alien Pests, Ministry of Agriculture and Rural Affairs/Hainan Key Laboratory for Detection and Control of Tropical Agricultural Pests, Haikou 571101, China

CITATION

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ABSTRACT

In this research, the inhibitory effect of 14 fungicides on *Phomopsis Heveicola* causing coffee leaf blight was investigated using mycelial growth method. The results showed that the EC_{50} values of 14 fungicides against mycelial growth ranged from 0.0680 to 258.2931 mg/L. Prochloraz, Prochloraz manganese salt, Difenoconazole, Carbendazim, Tebuconazole and Azoxystrobin had strong inhibitory activities on mycelial growth, and their EC_{50} values were 0.0680, 0.0696, 0.1923, 0.2360, 0.7630 and 0.8731 mg/L, respectively. Followed by Myclobutanil, Thiophanate-methyl, Pyrimethanil, Mancozeb, Propineb, Chlorothalonil and Enoylmorpholine, their EC_{50} values are: 2.2231 mg/L, 2.5048 mg /L, 3.5960 mg /L, 6.6887 mg /L, 8.0122 mg /L,8.2823 mg/L and 13.9420 mg/L. One fungicide with poor effect was Iprodione, EC_{50} values was 258.2931 mg/L The results of this research could provide scientific evidence for the effective control of coffee leaf blight, and more optional pesticides for utilization in the production practice of coffee industry.

Key words: *Phomopsis Heveicola*, Coffee leaf blight, Inhibitory fungicides, Fungicides screening indoor.

1. INTRODUCTION

Coffee is a major traded commodity for the developing world.[1] It was introduced to China over 100 years ago and now is an important cash crop in Yunnan and Hainan provinces [1-2]. Disease is among the prominent factors that constrain coffee production. The most significant and widespread diseases are rust (*Hemileia vastatrix*), Cercospora leaf spot (*Cercospora coffeicola*), Phoma leaf spot (*Phoma* spp.) and anthracnose and blister spot (*Colletrichum* spp.).

Phomopsis heveicola is an anamorphic stage of *Diaporthe tulliensis*. Disease incidence ranged in Yunnan and Hainan from 10-20% in the affected coffee plantings, and in extreme cases, 45% out of 200 trees were affected [3]. Symptomatic leaves initially exhibited small, reddish-brown, round or oval spots on the tip of leaves, subsequently expanding in size along the leaf margin, infected leaves eventually became wilted and dry. The pathogen affects coffee plant tissues such as leaf, stem and fruit, causing severe losses to coffee production.

Based on enhancing cultivation management measures and improving plant ability of disease resistance, prevention and control of coffee diseases are still dominant by chemical agents, and common fungicides include Prochloraz, Difenoconazole, Tebuconazole, Mancozeb, Chlorothalonil, Carbendazim etc.[4-11]. However, continuous and unreasonable use of chemical pesticides not only causes pesticide residue and environmental pollution, but also makes the pathogen produce resistance and declines control effect [12-14]. Therefore, it is of necessary to screen more and effective fungicides for reasonable mixing and rotation, to delay or reduce the production of drug resistance, prolong use life of fungicides, and finally reach the target of effectively preventing and controlling disease. To find out the effective fungicides against *P. Heveicola* experiment was conducted based on mycelial growth rate method, inhibition effect of 14 common fungicides on *P. Heveicola* causing coffee leaf blight was studied, which aimed to screen effective inhibitory fungicides, and provide scientific basis and test data for effective prevention and control of coffee leaf blight.

2. MATERIALS AND METHODS

2.1. Materials

Field survey was undertaken in Hainan and Yunnan during 2019. Leaves with typical symptoms were randomly collected from five coffee plantations in five counties and then subjected to mycological and pathological analysis. Initial identification of *P. heveicola* isolated from infected coffee leaves. The virulence of isolates was determined by pathogenicity test on coffee plants. 14 kinds of fungicides and their manufacturers were shown as Table 1.

Table 1. 14 kinds of fungicides, manufacturers and test concentration

Fungicide	Manufacturer	The selected concentration (mg/L)
97.2% Prochloraz WP	Guangdong Dafeng Plant Protection Technology Co., Ltd	1,0.5,0.25,0.1,0.05,0.01
80% Carbendazim WP	Hebei Angenuo Agrochemical Co., Ltd.	1,0.5,0.1,0.05,0.025,0.01
50%Prochloraz manganese salt WP	An Agricultural Sciences Co., Ltd.	1,0.5,0.1,0.05,0.025,0.01
50% Azoxystrobin WDG	Jiangsu Kesheng Group Co., Ltd.	1,0.5,0.1,0.05,0.025,0.01
95% Difenoconazole WP	Shandong Dongtai Agricultural Chemistry Co., Ltd.	1,0.5,0.1,0.05,0.025,0.01
40% Pyrimethanil WP	Hebei Angenuo Agrochemical Co., Ltd.	10,5,1,0.5,01
70% Thiophanate-methyl WP	Jiangsu Rotam Chemistry Co., Ltd.	10,5,1,0.5,0.1
97.3% Tebuconazole WP	Hebei Angenuo Agrochemical Co., Ltd.	1,0.5,0.1,0.05,0.025,0.01
80% Enoylmorpholine WP	Shenzhen Noposion Agrochemicals Co., Ltd.	50,10,5,1,0.5,0.1
40% Myclobutanil WP	Shaanxi Biaozheng Crop Technology Co., Ltd.	10,5,1,0.5,0.1
80% Mancozeb WP	Nantong DEYI Chemical Co., Ltd.	50,10,5,1,0.5,0.1
75% Chlorothalonil WP	Syngenta (Suzhou) Crop Protection Co., Ltd.	50,10,5,1,0.5,0.1
70% Propineb WP	Bayer Cropscience (China) Co., Ltd.	50,10,5,1,0.5,0.1
50% Iprodione WP	Shandong Zhongnongminchang Chemical Industry Co., Ltd.	500,400,300,200,100

2.2. Methods

2.2.1. Preparation of drug-containing PDA medium.

According to the result of preliminary test,14 fungicides were diluted into 5-6 gradient concentrations with sterile water. 1 mL of diluent was added into the conical flask containing 99 mL of 50℃ of sterilized PDA medium, and then set to a constant volume of 100 mL. After shaken evenly, the solution was poured into a petri dish (diameter 9 cm), and drug-containing PDA medium was prepared after solidification. Those added with sterile water used as blank control.

2.2.2. Mycelial growth rate method.

Under aseptic conditions, fungal cake was picked from colony edge of *P. heveicola* cultured for 5 d with a puncher (diameter 5 mm). the fungal cake was placed in the center of drug-containing medium. Each treatment replicated four times, and the PDA plate without fungicides was used as blank control. The plate was cultured at 25℃ under alternating light and dark(12h/12h) for 5 d, and colony diameter was measured by crossing method. The colony diameters of various treatment and inhibition rate of mycelial growth were calculated. The inhibition rate of mycelial growth was calculated according to formula (1):

Inhibition rate of mycelial growth (%) =
(Colony diameter in control-Colony diameter in treatment) / Colony diameter in control×100 (1)

2.2.3. Data processing

All data were statistically analyzed using DPS software. Using the logarithm of set mass concentration as the abscissa(x) and the probability of inhibition rate as the ordinate(y), the toxicity regression equation of each fungicide to evaluated strain y=a+bx, and median effective concentration (EC_{50} value) were obtained.

3. RESULTS AND ANALYSIS

3.1. Inhibitory effect on mycelial.

The inhibitory effects of 14 fungicides on mycelial growth of *P. Heveicola* were determined as shown in Table 2, the EC_{50} values of 14 fungicides against mycelial growth ranged from 0.0680 to 258.2931 mg/L. Prochloraz, Prochloraz manganese salt, Difenoconazole, Carbendazim, Tebuconazole and Azoxystrobin had strong inhibitory activities on mycelial growth, and their EC_{50} values were 0.0680, 0.0696, 0.1923, 0.2360, 0.7630 and 0.8731 mg/L, respectively. Myclobutanil, Thiophanate-methyl, Pyrimethanil, Mancozeb, Propineb, Chlorothalonil and Enoyl morpholine had relatively low inhibitory activity against mycelial growth, and their EC_{50} values are: 2.2231 mg/L, 2.5048 mg /L, 3.5960 mg /L, 6.6887 mg /L, 8.0122 mg /L,8.2823 mg/L and 13.9420 mg/L, respectively. One fungicide with poor effect was Iprodione, EC_{50} values was 258.2931 mg/L.

Table 2. inhibitory effects of 14 fungicides on mycelial growth *Phomopsis Heveicola*

Fungicide	Regression equation	Correlation coefficient （R）	EC_{50} value （mg/L）
prochloraz	y=0.9157x+6.0691	0.9274	0.0680
Prochloraz manganese salt	y=1.2929x+6.4961	0.9567	0.0696
Difenoconazole	y=0.841x+5.6021	0.9484	0.1923
Carbendazim	y=1.0388x+5.6515	0.9811	0.2360
Tebuconazole	y=1.1388x+5.1338	0.9809	0.7630
Azoxystrobin	y=1.6467x+5.0381	0.9739	0.8731
Myclobutanil	y=2.4421x+4.1527	0.9766	2.2231
Thiophanate-methyl	y=1.9778x+4.2113	0.9620	2.5048
Pyrimethanil	y=3.5143x+3.0467	0.9176	3.5960
Mancozeb	y=1.1021x+4.1679	0.9876	6.6887
propineb	y=1.1813x+3.9324	0.9388	8.0122
Chlorothalonil	y=0.6011x+4.4481	0.9318	8.2823
Enoylmorpholine	y=2.1909x+2.4929	0.9905	13.9420
Iprodione	y=1.5884x+1.1686	0.9612	258.2931

Table 3. inhibitory effects of ten types of fungicides on mycelial growth on *Phomopsis Heveicola*

Type of fungicide	Fungicide	EC_{50} value against mycelial growth(mg/L)
Benzimidazoles (MBCs)	Carbendazim	0.2360
Ergosterol inhibitors (SBIs)	Difenoconazole Prochloraz Prochloraz manganese salt Tebuconazole	0.1923 0.0680 0.0696 0.7630
Diformimides (DCFs)	Iprodione	258.2931
Strobilurins (QoIs)	Azoxystrobin	0.8731
Protective fungicides	Mancozeb Chlorothalonil	6.6887 8.2823
Triazole fungicides	Myclobutanil	2.2231
substitutive benzene fungicide	Thiophanate-methyl	2.5048
Phenylaminopyrimidine fungicides	Pyrimethanil	3.5960
Thiocarbamate fungicides	propineb	8.0122
Morpholine fungicides	Enoylmorpholine	13.9420

3.2. Inhibition characteristics of ten type of fungicides

The inhibitory effects of ten types of fungicides on mycelial grown of *P. Heveicola* were compared (Table 3). The results shown that Ergosterol inhibitors (SBIs), Benzimidazoles (MBCs) and Strobilurins (QoIs) had strong inhibitory activity against mycelial growth, Diformimides (DCFs) had weak inhibitory activity against mycelial grown.

4. CONCLUSION AND DISCUSSION

The genus *Diaporthe* (*Phomopsis* Sacc. & Harter) infect various agricultural and horticultural important crops and cause diseases such as damping off, leaf spots, blights, canker, dieback, wilt, root and fruit rots. causing huge yield and economic loss. Being primarily seed borne it also hinders import and export of germplasm and seeds [15-18]. Therefore, extensive characterization is required to diagnose and manage the disease. Chemical agent is still main means of current disease prevention and control. Coffee leaf blight caused by *P. Heveicola* is one of the major constraints in the production of coffee. To find out the effective fungicides against *P. Heveicola* experiment was conducted in-vitro evaluation of fungicides. The results revealed that Prochloraz, Prochloraz manganese salt, Difenoconazole, Carbendazim, Tebuconazole and Azoxystrobin had strong inhibitory activities on mycelial growth of *P. Heveicola,* Myclobutanil, Thiophanate-methyl, Pyrimethanil, Mancozeb, Propineb, Chlorothalonil and Enoyl morpholine had relatively lower inhibitory activity against mycelial growth, and their EC_{50} values are: 2.2231 mg/L, 2.5048 mg /L, 3.5960 mg /L, 6.6887 mg /L, 8.0122 mg /L,8.2823 mg/L and 13.9420 mg/L, respectively. Iprodione with poor effectivity.

From the mechanism of action, MBCs mainly inhibit cell mitosis, SBIs mainly inhibit the synthesis of biofilms; and QoIs ,Protective fungicides, substitutive benzene fungicide, Phenylamino, pyrimidine fungicides and Thiocarbamate fungicides are respiratory inhibitors while protective fungicides act on multiple sites. Triazole fungicides can protect plants by affecting the biosynthesis of fungal sterols and destroying the function of cell membrane. Morpholine fungicides have strong internal absorption, protective and therapeutic effects can inhibit the biosynthesis of ergosterol. DCFs are signal transduction inhibitors that increase intracellular osmotic pressure and eventually lead to cell death. Our research results showed that MBCs, SBIs and QoIs had strong inhibitory activities on mycelial growth. However, DCFs had poor inhibitory activity on mycelial growth. Therefore, these fungicides such as Carbendazim, Difenoconazole, Prochloraz, Prochloraz manganese salt, Tebuconazole, Azoxystrobin, Mancozeb, Chlorothalonil, Myclobutanil, Thiophanate-methyl, Pyrimethanil, pro-

pineb and Enoylmorpholine should applied in growth stage of *P. Heveicola.* However, the bactericidal spectrum and bactericidal characteristic of the same type of fungicides may be greatly different among different varieties, the bactericidal varieties determined in this test could not represent all the varieties of this type of fungicides. The inhibitory characteristics of the other varieties of fungicides against *P. Heveicola* still need to be further determined.

Due to different application patterns and environment, indoor toxicity test results were often different from field test results. The indoor toxicities of 14 fungicides against *P. Heveicola* were determined in the test, and the field control efficacy should be further test and verified. The inhibitory effects of fungicides on mycelial growth were determined in the test, and the effect on spore germination and sporulation still need to be further studies. In addition, the toxicity was determined by finished preparations, which was more helpful to guide field application than active compound determination. However, fillers and auxiliars in preparation processing may affect determination results, and the influence of different preparation processing technologies on *P. Heveicola* need to be further determined

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