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IDENTIFICATION OF CONTROL AGENTS AGAINST MELON WILT DISEASE IN LABORATORY AND FIELD IN NE-CHINA

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Abstract

The pathogenic bacteria of *Fusarium oxysporum* were obtained through tissue separation. The inhibition of 13 fungicides on growth of *Fusarium oxysporum* was determined in laboratory. The results showed 30% Trimethoprim WP and 10% difenoconazole WG significantly inhibited the growth of *Fusarium oxysporum* at EC_{50} of 0.0025μg / mL and 0.8120μg / mL, respectively. Whereas, 99% hymexazol TF,50% chlorothalonil WP, 70% thiophanate-methyl WP, 50% thiram WP, fairly inhibited the growth at EC_{50} <10μg / Ml. The 50% iprodione WP, 50% procymidone WP, 65% Oxadixyl WP, 15% triadimefon WP, 70% mancozeb WP, 10% polyoxin WP, and 65% Tiezene WP provided less effects. The 30% trifloxystrone WP and 10% difenoconazole WG were tested in field due to their well performance in laboratory.The control efficacy was significantly higher than that of 30% Rui Miaoqing that functioned as control.

Key words: Melon Fusarium wilt; Tissue separation; Bactericide; Growth rate; Field control.

Introduction

Melon blight, also called dead seedling, dead vine, wilting disease and vine cut disease, has been a main disease during melon growth and a world-wide soil-borne disease. The disease can occur throughout the growth of melon, causing the whole plant wilting-dead or rot in the soil and failing to earth. Fusarium wilt has a strong vitality in the soil, mainly in the mycelium, chlamydospores and sclerotia, and could not be decomposed with fertility, and thus become the main source of infection in the next year. The case can survive 5 to 6 years (Liu *et al.*, 2007; Chen *et al.*, 2015).

Melon as an annual creeping herb of Cucurbitaceae, is widely cultivated worldwide. In recent years, with the rapid development of cultivation facilities in China, melon planting area has been increasing, but the occurrence of melon wilt disease is aggravating, and the incidence rate reaching 50% ~ 70% now (Liu *et al*., 2010).The disease has become the impact of melon quality and melon blight has become one of the most serious diseases in Jilin, Liaoning, Shandong and other provinces of China (Zhao *et al*., 2011). The disease has caused tremendous losses to melon farming industry, which is beyond what the melon farmers can accept.

Fusarium wilt is caused by *Fusarium oxysporum*. Since the first report of melon blight by Smith in 1894 (Wang *et al*., 2013), scientists began to work on the blight. At present, melon blight has been reported to widely occur in the Americas, Europe, Asia and Africa. Although researchers had done a great deal of work for the prevention and control of the disease by employing fungicides, disease-resistant breeding, crop rotation, grafting, high-temperature sterilization, and other means, but the control effect was not ideal (Xu *et al*., 2014).The research on melon wilt in China mainly is concentrated on the comprehensive prevention in terms of the screening fungicides for the disease control. At present, the disease control is fairly relied on chemicals (Li *et al*., 2014; Stanković *et al*., 2015).

Melon is one of the eight major fruits that is sweet, fragrant, and rich in sugar and starch, and has a small amount of protein, minerals and other vitamins. The summer melon can eliminate heat, polydipsia, and is conducive to the human liver. China is a country with a big production and consumption of melons. However, with the development of planting area, the accumulation of soil bacteria in greenhouses and plastic sheds has been gradually increased, causing large spread of melon wilt, which has caused serious problems in melon farming in China with great economic loss (Qiao *et al*., 2013). At present, the control of melon blight is still rely on chemical agents having highly toxic-fungicides that easily lead to environmental pollution and pesticide residues, there is no efficient biological pesticides for the use.

In case of disease-resistant varieties occurrence, chemical control is also an important measure to control the epidemic of melon wilt, and is favored by the farmer due to its high anti-efficiency, quick response, broad spectrum of sterilization, and low cost. In this study, we obtained *Fusarium oxysporum* sp. pathogenic bacteria in the surrounding area of Jilin Province by tissue separation. There were 13 fungicides identified and they were tested against the disease in terms of growth rate. The experiment was to screen out effective agents for the prevention and treatment of the disease. Furthermore, the identified chemical agents were tested in field to ensure the ideal agents that can be used in the future production on larger scale.

Materials and Methods

Bacteria strains: *Fusarium oxysporum* used in this experiment was collected from the surrounding area of Changchun and was isolated, identified and preserved in Plant Pathology Laboratory of Jilin Agricultural University.

Test agents: Thirteen fungicides (Zhao *et al*., 2006; Wang *et al*., 2013; He *et al*., 2014) were tested as shown in Table 1.

Table 1. Fungicides tested in this study in 2017 year.

Numbering	Pharmacy name	Manufactory
1	30% Trimethoprim WP	Tak Keung Biological Co., Ltd.
2	10% Difenoconazole WG	Jiangsu Feng Deng Crop Protection Co., Ltd.
3	99% Hypogeacean TC	Yantai Xinrun Fine Chemical Co., Ltd.
4	50% Chlorothalonil WP	Limin Chemical Co., Ltd.
5	70%Thiophanatemethyl WP	Beijing Mause Technology Co., Ltd.
6	50% Thiram WP	Hebei Guanlong Agrochemical Co., Ltd.
7	50% Iprodione WP	Jiangxi Wo Yi Chemical Co., Ltd.
8	50% Procymidone WP	Sumitomo Chemical Co., Ltd.
9	65% Oxadixyl WP	Syngenta Crop Protection Limited
10	15% Triadimefon WP	Sichuan Guoguang Chemical Co., Ltd.
11	70% Mancozeb WP	Limin Chemical Co., Ltd.
12	10% Polymyxin WP	Japan Research Pharmaceutical Co., Ltd.
13	65% Tiezene WP	Shaanxi Road on the grid Bioscience Co., Ltd.

Pathogen isolation, purification, preservation: Tissue isolation method was used for the isolation of melon wilt strains (Fang, 1998; Li *et al*., 2007). The melon wilt strains were rinsed with tap water, and then with (75% alcohol 10s, 0.1% mercuric chloride 1 min, sterile water rinse 2 to 3 times) and was transferred into potato dextrose culture medium, cultured at 28°C for 2 ~ 3 days. After growing the mycelium on the plate, single spore was isolated and spores were picked from the colonies. Otherwise the mycelium was transformed into potato dextrose medium without antibiotics (PDA) medium for repeated purification. The purified strain was transplanted onto the PDA slant and was placed in a refrigerator at 4°C for later use.

Growth rate measurement: Mycelial growth rate method was used to determine the inhibition of fungicide on growth of *Fusarium oxysporum* sp. (Li *et al*., 2004; Zhou *et al*., 2006; Yang *et al*., 2010).Each of the above-mentioned agents was diluted with sterile water and 6 concentration of the employed fungicides was employed.The concentrations involved were: 1×10^4, 1×10^3, 1×10^2, 1×10^1, 1×10^0, and 1×10^{-1}mg/L, respectively. The PDA medium was mixed with above individual dosages at ratio of 1: 9. With a diameter of 8mm Pipette tip, and at the edge of bacteria3d Melon Fusarium bacteria colonies were obtained and inoculated to PDA either containing test drug or PDA containing non-drug. Each individual drug-concentration functioned as a treatment with three replications at 26-27°C for 3d-7d. With Cross method, the colony diameter was measured, compared with the control colony diameter, to identify the ideal agents for well inhibition of bacterial strain growth.

The inhibition rate of the colony is calculated as follows:

$$\text{Growth inhibition (\%)} = \frac{\text{Control colony diameter - Treated colony diameter}}{\text{Control colony diameter - Bacterial cake diameter}} \text{ x 100 \%}$$

Data analysis by the Excel. The inhibition rate of growth was calculated and converted into the probability of dependent variable (y), and the logarithm value (x) of the drug concentration was taken as the logarithmic value (x) bx. According to the linear regression equation, when taking y = 5, the negative logarithm of the value of x is the value of EC_{50}. When y = 6.28, the negative logarithm of the value of x is the value of EC_{90}.

Field test: At the experimental base of Jilin Agricultural University, the tests were conducted at a place which was rich in water and fertilizer. The experiment was designed with a total of 6 treatments and each individual had 3 replicates. A total of 18 plots were employed with each having an area of 10 m^2. The fungicides were applied on August 18, 2017, and 30 % Rui Miao-Qing and water were applied as control.

Experiment design: The experiment was conducted according to the pesticide field efficacy test guidelines. The incidence of melon wilt disease was investigated 7 days after the pesticide application. Ten plants were selected diagonally in the plot to investigate all the disease-infecting plants. The disease status was graded based on the percent of the disease area of each plant. Grading investigation and prevention effect calculation formula is as follows:

$$\frac{\text{Disease index} = \Sigma\,(\text{number of diseased plants} \times \text{representatives of all levels}) \times 100}{\text{The total number of plants investigated} \times \text{the highest representative}}$$

$$\frac{\text{Relative control effect} = \text{control disease index} - \text{treatment of disease index} \times 100\%}{\text{Control disease index}}$$

Results

Isolation and preservation of pathogens: *Fusarium oxysporum* sp. were isolated by tissue isolation and stored on PDA slant culture medium.The growth of *Fusarium oxysporum* sp. on PDA containing drug was inhibited, and the antibacterial effect was better. Overall, all the agents provided bigger inhibition with increasing fungicide- concentration (Table 2). Among them, 30% trifloxisol WP and 10% difenoconazole WG had higher virulence to *Fusarium oxysporum* f. Sp., at 0.0025μg / mL and at EC_{50} of 0.8120μg / mL, respectively. This was followed by 99% hymexazol TF, 50% chlorothalonil WP, 70% thiophanate-methyl WP, and 50% thiram WP, at the EC_{50} of 3.3368, 4.8289, 7.1350, and 9.3081μg / mL. The 50% iprodione WP, 50% Procymidone WP, 65% Oxadixyl WP, 15% triadimefon WP, 70% mancozeb WP, 10% polyoxin WP, and 65% Tiezene WP provided less effect.

Field test: In order to further verify the laboratory test results, field control was conducted. Effect of the two kinds of fungicides in field were tested, the fungicides of 30% Ruimiao Qing AS worked as treatment and water as a control (Table 3). According to the test results in Table 3, the field efficacy of 30% fluraziconazole WP and 10% difenoconazole WG was significantly higher than that of the of 30% Meloncontrol.

Table 2. Inhibition effects of 13 fungicides to *Fusarium oxysporum* Melonis.

Numbering	Fungicides	Toxicity regression equation	Correlation coefficient	EC_{50}(μg/ mL)	EC_{90}(μg/ mL)
1	30%Trimethoprim WP	Y = 7.9343-0.1480x	0.9829	0.0025	159.6379
2	10% Difenoconazole WG	Y = 7.4180-0.1724x	0.9952	0.8120	1360.4075
3	99% Hypogeacean TF	Y = 8.4506-0.2736x	0.9797	3.3368	358.8635
4	50% Chlorothalonil WP	Y = 7.0877-0.1706x	0.9912	4.8289	8774.2962
5	70% Thiophanatemethyl WP	Y = 7.2004-0.1857x	0.9409	7.1350	7033.70416
6	50% Thiram WP	Y = 7.7954-0.2413x	0.9858	9.3081	1873.1093
7	50% Iprodione WP	Y = 7.6917-0.2651x	0.9146	38.8627	4862.3795
8	50% Procymidone WP	Y = 6.1862-0.1202x	0.9692	51.7173	2182955.1917
9	65% Oxadixyl WP	Y = 6.7627-0.1829x	0.9583	65.2274	71421.7251
10	15% Triadimefon WP	Y = 6.3167-0.1523x	0.9675	176.0609	785665.2497
11	70% Mancozeb WP	Y = 5.9937-0.1428x	0.7485	950.7986	7424858.3108
12	10% Polymyxin WP	Y = 5.5813-0.1386x	0.8197	14093.1054	167931097.0046
13	65% Tiezene WP	Y = 5.2302-0.0647x	0.9630	28477.3908	11216498738576

Table 3. The control efficacy of 2 fungicides in field in 2017 year.

Fungicides	Application dose ($ga.i/hm^{-2}$)	Condition index	Control effect (%)	Significant difference 5%	1%
30% Trimethoprim WP	420	4.02	82.12	a	A
10% Difenoconazole WG	420	5.41	80.69	ab	AB
30% Rui Miao Qing AS	420	10.29	73.23	bc	B
CK	water	40.54			

Discussion

Thirteen fungicides were tested for indoor virulence using the growth rate method and 30% trifloxisol WP and 10% difenoconazole WG were identified due to its higher virulence. The pathogens were more sensitive to 30% triflumizole WP and 10% benzene Methotrexate WG at EC50s of 0.0025 μg / mL and 0.8120 μg / mL, respectively. Based on the determination of the virulence of *Fusarium oxysporum* f. sp., the above two agents should be screened as they may be possible agents for the control of melon wilt in the field (Zhao *et al*., 2010; Xu, *et al*., 2017; Gunavathy *et al*., 2018).

The results of field control showed that the 30% trifloxisol WP and 10% difenoconazole WG 2 provided better control effect than that of 30% Miao Qing AS. Through this indoor antibacterial activity determination and field efficacy test, efficient and low toxicity *Fusarium oxysporum* fungicides were identified and here provided a scientific basis for the prevention and treatment of melon Fusarium wilt (Yang *et al*., 1999; Li *et al*., 2007; Xu *et al*., 2014).

In this study, only the growth rate method was used to determine the virulence of *Fusarium oxysporum* sp. Grisea. The influence of spore germination and the mode of action during the experiment were essential to be further studied in the future. In addition, the tested fungicides used here is more efficient than the original drug, which may have some impact on additives and fillers on the bacteria. This may be gradually improved in the future study.

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