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INHIBITION OF FUNGICIDE ON *SCLEROTINIA SCLEROTIORUM* OF CUCUMBER AT DIFFERENT GROWTH STAGES AND FIELD CONTROL EFFECTS

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Abstract

In order to screen high-efficient fungicides against *Sclerotinia* rot of cucumber, the growth rate inhibition method was used to determine the effects of 10 fungicides on the silk growth and *sclerotia* formation of *Sclerotinia* rot of cucumber, and the virulence regression equation was constructed to calculate the EC_{50} value. On the basis of indoor experiments, four kinds of fungicides with good inhibition effect were selected for field efficacy trials and their effects on controlling cucumber s*clerotinia* rot in the field were evaluated. The indoor results show that: The growth of *Sclerotinia sclerotiorum* silk was highly inhibited by 200g/L fluoroacyl-hydroxy-benzotriazole (SC), 45% prochloraz (EW), 25% imazalil-fludioxonil (SE), 38% Oxazolidinyl bacteria. The growth of *Sclerotinia sclerotiorum* nucleus was highly inhibited by 38% azolyl-boscalid (SE), 50% boscalid (WG), 200g/L Benzene propiconazole (EC) and 25% imazalil-fludioxonill (SE). Two fungicides, 25% imazalil-fludioxonil (SE) and 38% Oxazolidinyl bacteria (SE), could inhibit the growth of both hyphae and sclerotium. The results of field efficacy trials showed that: The field efficacy of 45% prochloraz (EW) and 200g/L fluoroacyl-hydroxy-benzotriazole (SC) was significantly higher than that of the control 50% carbendazim (WP). The field efficacy of 25% imazalil-fludioxonil (SE) and 38% azolyl-boscalid (SE) was not as significant as that of the control. This experiment provided a theoretical and practical basis for the fungicide control of *Sclerotinia* rot of cucumber in production.

Key words: *Sclerotinia sclerotiorum*; Growth rate method; Indoor toxicity determination; Field.

Introduction

Cucumber is one of the vegetables that people eat all the year round (Li, 2020). The Cucumber *Sclerotinia* rot caused by *Sclerotinia sclerotiorum* (lib.) De bary is one of the devastating diseases in cucumber cultivation in the protected field in northern China (Pan *et al*., 2006). The main reasons why *Sclerotinia sclerotiorum* is difficult to control are as follows: firstly, as the primary infection source of disease cycle and the main form of pathogen survival, *Sclerotinia* can survive for 8 years in adverse environment (Clarkson *et al*., 2007); Secondly, *sclerotia* can germinate to form apothecium, release ascospores, and spread for thousands of meters along with the airflow, and then generate hyphae to infect stems, leaves and fruits of the host, which is the main reason for the large-scale occurrence of *Sclerotinia sclerotiorum* (Mila & Yang, 2008; Hussain *et al*., 2019). Finally, the *sclerotia* can also germinate and generate hyphae to directly infect the crop roots, leading to root or stem rot and, in severe cases, the death of the host plant (Azevedo *et al*., 2016). According to the survey, it generally takes only 3 to 4 years for these diseases to develop from sporadic ones to widespread ones, with the diseased plant rate reaching 100% and the yield reduction exceeding 50%. Low temperature, high humidity or rainy early spring or late autumn was conducive to the occurrence or prevalence of *Sclerotinia* rot of cucumber (Ji, 2016).

Chemical control was mainly adopted for *Sclerotinia* rot of cucumber in production, but no special agent for controlling *Sclerotinia* rot of cucumber was registered in China at present. Fungicides against *Sclerotinia* rot of cucumber mainly include thiophanate-methyl, dimethachlon, procymidone, iprodione and difenoconazole (Shi *et al*., 2020). It was reported that the benzimidazole fungicides carbendazim and thiophanate-methyl were currently used in many areas, resulting in poor control effect. The residual amount on the fruit also exceeded the limit value stipulated by the state (Lv *et al*., 2014; Zhu *et al*., 2008). However, spraying with such imide fungicides as dimethachlon and procymidone showed good control effects on *Sclerotinia* rot of cucumber (Kuai & Wu, 2012; Wang *et al*., 2010). The results showed that triazole fungicides such as hexaconazole had high toxicity for inhibiting the mycelial growth of *Sclerotinia* rot of cucumber, but little effect on the formation and germination of *sclerotia*. Although the single weight of *sclerotia* was decreased by increasing the concentration of fungicides, due to the certain inhibition effect of the fungicides on the growth of plants, and the use of a large amount of pesticides, the drug resistance of plant diseases and insect pests was enhanced, and the harm degree was significantly increased. Pesticide residues on melon strips exceeded the standard, which directly endangered human health and easily caused environmental pollution (Luo *et al*., 2001).

In this study, 10 fungicides with inhibitory effect on *Sclerotinia* rot of cucumber were selected and the inhibition of fungicides on the growth of hypha and sclerotia of *Sclerotinia sclerotiorum* was determined by growth rate method. On the basis of indoor bacteriostasis test, four kinds of fungicides with obvious effect were selected to evaluate the field control effect, which provided a theoretical basis for the control of *Sclerotinia* rot of cucumber.

Materials and Methods

The test strain: *Sclerotinia sclerotiorum* was isolated from and stored in the Bacterial Disease Laboratory of School of Plant Protection, Jilin Agricultural University.

Test preparations: There were 10 kinds of test preparations, as shown in Table 1.

Table 1. Preparation, active ingredients and manufacturers of fungicides tested.

No.	Test fungicides	Manufacturer
1.	25g/L Fludioxonil FS	Zhengda Nantong crop protection co., ltd.
2.	38% Oxazolidinyl bacteria SC	Zhejiang Zhongshan Chemical Group Co., Ltd.
3.	25% Pyrimidine WP	Zhejiang Gramineae Technology Co., Ltd.
4.	25% Imazalil fludioxonil SE	Yifan Biotechnology Co., Ltd.
5.	225g/L Iprodione SC	Zhejiang Tianfeng Biological Science Co., Ltd.
6.	200g/L Fluoro-hydroxy-benzoxazole SC	Switzerland Syngenta Crop Protection Co., Ltd.
7.	1% Phenazine-1-carboxylic acid SC	Shanghai Nongle Biological Products Co., Ltd.
8.	50% Boscalid WG	BASF Europe Ltd.
9.	200g/L Benzene propiconazole EC	Yifan Biotechnology Group Co., Ltd.
10.	45% Prochloraz EW	Sichuan lier crops technology co., ltd

Fungicide on Cucumber *sclerotinia* rot hypha growth inhibition test: Using growth rate method (Chen *et al*., 2019), each drug was formulated into five drug solutions with mass concentration of 1×10^4, 1×10^3, 1×10^2, 1×10, 1×10^0 and 1×10^{-1}mg/L, respectively. The drug-containing plate medium was prepared according to the ratio of liquid medicine to PDA1: 10, and the plate mixed with equal volume of sterile water and PDA was taken as the control (Wang *et al*., 2019). After the plate was condensed, the *Sclerotinia sclerotiorum* cake cultured on PDA for 7 days was punched with an 8mm diameter hole punch and inoculated in the center of the drug-containing plate, and three groups of replicates were set. After the control grew to 6cm (the petri dishes with the diameter of 9cm were used in this study), the colonies of each treatment were measured by cross method and the inhibition rate of each treatment on hypha growth was calculated after being cultured in an incubator at 25℃ for 5-7 days.

Inhibition of fungicide on *sclerotia* growth of *Sclerotinia sclerotiorum*: The 10 preparations were prepared into the liquid medicine with the above five mass concentrations and the drug-containing plate was made, with the plate mixed with equal volume of sterile water and PDA as the control. After the plate was condensed, Inoculating *Sclerotinia* rot of cucumber with the same size was inoculated and three groups of replications were set. The samples were inverted and cultured in an incubator at 25℃ for 5-7 days. After the control grew to 6cm, the colony diameters of each treatment were measured and the inhibition rate was calculated (Ma *et al*., 2020).

Field control effect of fungicides on *Sclerotinia sclerotiorum*

Experimental design: Four fungicides with good indoor screening effect were selected for field efficacy trials (Feng *et al*., 2013) in the experimental base of Jilin Agricultural University. Six treatments were designed and repeated three times in 18 residential areas, with an area of $10m^2$ per residential area. All the residential areas were arranged in a random combination. The fungicides were applied to the initially infected cucumber plant on July 21, 2021, with 50% carbendazim WP and clean water as controls, according to their reasonable use amounts.

Field investigation method: The experimental investigation was conducted according to the guidelines for field efficacy trials of pesticides. The incidence of *Sclerotinia* rot of cucumber was investigated 7 days after application, and 10 plants were investigated at 5 random points in the plot. All diseased leaves of each plant were investigated, and the disease was graded according to the percentage of the diseased area of each leaf. The criteria for disease grading and the calculation formula of control effect were as follows:

Classification criteria: Level 1: formation of a water-stained yellowish-brown lesion with a diameter of 5-9 mm; Grade 2: water-stain-like yellowish-brown lesion with a diameter of 10-14 mm was formed; Grade 3: water-stained yellowish-brown lesion with a diameter of 15-19 mm was formed; Grade 4: A water-stained, yellowish-brown lesion ≧ 20mm in diameter was forme.

Statistical analysis: The experimental data were analyzed by Excel software, and the regression equation of toxicity of each agent to hypha and sclerotia was constructed. The EC_{50} value and the correlation coefficient r were calculated.

Effect of different fungicides on hypha growth: Table 2 shows that EC_{50} values different kinds bactericide vary greatly. The EC_{50} values of 10 fungicides to hypha from large to small are as follows: Phenazine-1-carboxylic acid (1%, SC) > Pyrimidine (25%, WP) > Boscalid (50%, WG) > Benzene propiconazole (200g/L, EC) > Fludioxonil (25g/L, FS) > Iprodione (225g/L SC) > Oxazolidinyl bacteria (38%, SC) > Imazalil fludioxonil (25%, SE) > Fluoro-hydroxy-benzoxazole (200g/L, SC) > Prochloraz (45%, EW) (Table 2). Prochloraz (45%, EW) has the strongest toxicity, and its EC_{50} value is 0.0144mg/L; Secondly, Fluoro-hydroxy-benzoxazole (200g/L, SC), its EC_{50} value is 0.0442mg/L.

$$\text{Disease parameter} = \frac{\Sigma\text{ (Parameter of damaged leaf x Value of various damaging levels)}}{\text{Tested leaf number x Value of the highest damage}} \text{ X 100}$$

$$\text{Control efficacy} = \frac{\text{(Disease parameter in CK – Disease parameter in treatment)}}{\text{Disease parameter in CK}} \text{ X 100}$$

Table 2. Effects of fungicides on the hypha growth of *sclerotiorum*.

S. No.	Test fungicides	Virulence regression equation	R	EC_{50} (mg/L)	EC_{90} (mg/L)
1.	25g/L Fludioxonil FS	Y=11.3785-0.5415X	0.9333	7.6600	81.4382
2.	38% Oxazolidinyl bacteria SC	Y=11.3785-0.5415X	0.9275	2.1450	29.5696
3.	25% Pyrimidine WP	Y=9.7731-0.4535X	0.9295	26.8525	451.4684
4.	25% Imazalil fludioxonil SE	Y=11.0983-0.4321X	0.8750	0.7430	14.3687
5.	225g/L Iprodione SC	Y=11.7706-0.5669X	0.9193	6.5037	62.1949
6.	200g/L Fluoro-hydroxy-benzoxazole SC	Y=9.8532-0.2866X	0.9099	0.0442	3.8481
7.	1% Phenazine-1-carboxylic acid SC	Y=9.9597-0.8635X	0.8660	3203.6680	14195.5208
8.	50% Boscalid WG	Y=9.7667-0.432X	0.9888	16.1206	312.1087
9.	200g/L Benzene propiconazole EC	Y=11.3137-0.5648X	0.9651	13.9606	134.6484
10.	45% Prochloraz EW	Y=9.7993-0.2658X	0.9331	0.0144	1.7757

Table 3. Effects of different fungicides on *Sclerotinia sclerotiorum* growth in cucumber.

S. No.	Test fungicides	Virulence regression equation	R	EC50 (mg/L)	EC90 (mg/L)
1	25g/L Fludioxonil FS	Y=10.7061-0.4059X	0.8734	0.7839	18.3615
2	38% Oxazolidinyl bacteria SC	Y=9.9387-0.3311X	0.9783	0.3330	15.8939
3	25% Pyrimidine WP	Y=10.0519-0.5381X	0.8281	83.7321	903.4649
4	25% Imazalil fludioxonil SE	Y=11.1286-0.4359X	0.8782	0.7833	14.7639
5	225g/L Iprodione SC	Y=11.5938-0.5602X	0.9396	7.7288	75.9363
6	200g/L Fluoro-hydroxy-benzoxazole SC	Y=10.8433-0.479X	0.9746	5.0302	72.8154
7	1% Phenazine-1-carboxylic acid SC	Y=8.5328-0.4115X	0.9376	186.9788	4193.6165
8	50% Boscalid WG	Y=8.1745-0.2685X	0.9234	7.3179	861.2678
9	200g/L Benzene propiconazole EC	Y=10.8005-0.4824X	0.9531	5.9970	85.1694
10	45% Prochloraz EW	Y=10.7179-0.4212X	0.9063	1.2737	26.5893

Experimental results and analysis

The inhibition rate of that same bactericide with different concentration on the hypha growth of the *sclerotinia sclerotiorum* is obviously different. Among the 10 fungicides tested, the suspension concentrate of Fluoro-hydroxy-benzoxazole (200g/L, SC) and the Prochloraz (45%, EW) emulsion in water had the best inhibition effect on the silk growth of *Sclerotinia sclerotiorum*; under the condition of the lowest concentration (0.1µg/mL), the hypha inhibition rates were 70.30% and 76.18%, respectively. The suspension concentrate of Oxazolidinyl bacteria (38%, SC) and that suspension emulsion of Imazalil fludioxonil (25%, SE) had good inhibition effect on the silk growth of *Sclerotinia sclerotiorum*, and the inhibition rate was 79.2%-82. 29% at a low concentration (1µg/mL). Among all the treatment concentrations, Phenazine-1-carboxylic acid (1%, SC) had the worst antibacterial effect. Under the condition of the highest concentration (10000µg/mL), the antibacterial rate of Phenazine-1-carboxylic acid (1%, SC) was 94.94%, and it had no inhibition effect on *Sclerotinia sclerotiorum* at other concentrations. The inhibition rates of the other nine fungicides tested at the highest concentration were 100%, and they had different antibacterial effects at low concentrations (Table 2).

Effects of different fungicides on the growth of *Sclerotia*: Table 3 shows that EC_{50} values different kinds bactericides vary greatly. The EC_{50} values of the ten fungicides for the growth of *Sclerotinia* ranged from high to low as follows: Phenazine-1-carboxylic acid (1%, SC) > 25% Pyrimidine WP > Iprodione (225g/L, SC)> Boscalid (50%, WG) > Benzene propiconazole (200g/L, EC) > Fluoro-hydroxy-benzoxazole (200g/L, SC) > Prochloraz (45%, EW) > Fludioxonil (25g/L, FS) > Imazalil fludioxonil (25%, SE) > Oxazolidinyl bacteria (38%, SC) (Table 3). The most toxic agent was Oxazolidinyl bacteria (38%, SC) and the EC50 value was 0.3330 mg/L. This was followed by Imazalil fludioxonil (25%, SE) with an EC50 of 0.7833 mg/L.

There were significant differences in the inhibition rate of the same fungicide at different concentrations on the growth of *sclerotia*. Among the 10 fungicides tested, Oxazolidinyl bacteria (38%, SC) and Boscalid (50%, WG) had the best inhibitory effect on the growth of *Sclerotinia*, and they inhibited the growth at the lowest concentration (0.1µg/mL), while the other 8 fungicides had no inhibition at the lowest concentration. The suspension seed-coating agent of Fludioxonil (25g/L, FS) and the Prochloraz (45%, EW) emulsion in water had good inhibition effect on the nuclear growth of *sclerotinia*, and the inhibition rate was 83.49%-94.42% under the condition of relatively low concentration (1µg/mL). Among all the treatment concentrations, Phenazine-1-carboxylic acid (1%, SC) exhibited the worst antibacterial effect, and the antibacterial rate reached more than 90% only at the highest concentration (10000µg/mL) (Table 3).

Table 4. Field efficacy of four fungicides against *Sclerotinia sclerotiorum*.

Test fungicides	Dosage of preparation ($ga.i/hm^{-2}$)	Disease indes	Control Efficacy (%)	Significance	
				5%	1%
45% Prochloraz EW	550	2.42	89.14	a	a
200g/L Fluoro-hydroxy-benzoxazole SC	420	3.16	86.79	a	ab
25% Imazalil fludioxonil SE	510	5.45	74.41	ab	b
38% Oxazolidinyl bacteria SC	450	673	70.32	c	c
50% carbendazim WP	480	8.12	78.56	c	c
CK	Water	20.41	-		

Field efficacy test results: The field efficacy trials of four fungicides with good indoor screening effect were conducted. The fungicides carbendazim (50%, WP) commonly used in production and clean water were selected as the control. The results are shown in Table 4.

According to the test results in Table 4, the field efficacy of prochloraz (45%, EW) and Fluoro-hydroxy-benzoxazole (200g/L, SC) was significantly higher than the field efficacy of the control carbendazim (50%, WP). It was recommended to use these two fungicides to control *Sclerotinia* rot of cucumber in the field. The field efficacy of Imazalil fludioxonil (25%, SE) and Oxazolidinyl bacteria (38%, SC) was not as good as the control and they were not recommended to be used in the field.

Discussions

Sclerotinia sclerotiorum is a highly epidemic and destructive disease that can harm cucumber, tomato and eggplant and is one of the major diseases in greenhouse cucumber production (Chen *et al*., 2007). In recent years, with the continuous expansion of the planting area of protected vegetables and the improvement of multiple cropping index, the damage of *Sclerotinia sclerotiorum* is becoming increasingly serious. Cucumber, as the main bulk vegetable, is an important economic vegetable crop and is favored by people. Chemical control has always been the most commonly used and main method taken by people to control *Sclerotinia* rot of cucumber, with the advantages of rapid and efficient, not subject to geographical and time constraints, convenient application, etc. However, common fungicides only inhibited the mycelial growth, but had no significant inhibition on the formation and germination of *sclerotia*, which could not fundamentally control the occurrence of the disease. Therefore, it was particularly important to screen out the fungicides that were effective for both to meet the actual production needs.

In this study, single dose of 10 fungicides was tested for indoor toxicity and 2 fungicides were screened out for field control of *Sclerotinia* rot of cucumber through field trials. Due to the limitation of long-term repeated use of a single chemical fungicide (An *et al*., 2021), attention should be paid to the alternate use of fungicides to avoid the development of drug resistance (Hui *et al*., 2021). Currently, our effective method by the selected fungicides has provided a better choice in practice with more potential application according to recent reports (Munir *et al*., 2019), But further research is needed on whether the compound agent between chemical agents and the compound agent between chemical agents and plant-derived agents have synergistic effect and control effect in the field (Zou *et al*., 2021).

Conclusions

In this experiment, 10 kinds of fungicides were screened and their control effects on *Sclerotinia* rot of cucumber were tested in laboratory. The results showed that 10 fungicides had different degrees of inhibition on the growth of *Sclerotinia sclerotiorum* silk and *sclerotia* of cucumber. The most potent inhibitor against *Sclerotinia sclerotiorum* silk growth was prochloraz (45%, EW), and the next most potent inhibitor was Fluoro-hydroxy-benzoxazole (200g/L, SC). The most potent inhibitor against *Sclerotinia sclerotiorum* nuclear growth was Oxazolidinyl bacteria (38%, SC), followed by Imazalil fludioxonil (25%, SE). The growth of *Sclerotinia sclerotiorum* silk and *sclerotia* of cucumber was inhibited by the two fungicides Imazalil fludioxonil (25%, SE) and Oxazolidinyl bacteria (38%, SC)at different concentrations. The field trials of the above four fungicides with better efficacy showed that the field antibacterial effects of prochloraz (45%, EW) and Fluoro-hydroxy-benzoxazole (200g/L, SC) were significant, which were recommended for field use.

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