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Sensitivity of *Sclerotinia sclerotiorum* to bixafen and its mixtures in Henan Province

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Abstract: Sclerotinia stem rot is a destructive disease caused by the fungus *Sclerotinia sclerotiorum*, and it occurs in oilseed rape and many other broadleaf crops. The chemical control with fungicides is usually applied in fields. As a kind of succinate dehydrogenase inhibitors (SDHIs), bixafen showed higher efficiency in inhibiting mycelial growth. To establish the baseline sensitivity of *S. sclerotiorum* to bixafen in Henan Province, 119 isolates of *S. sclerotiorum* were obtained from diseased stems of oilseed rape collected from fields in different geographical regions in Henan Province, China, in 2015 and 2016, and their sensitivity to bixafen was determined based on mycelial growth rate method. The results showed that the 50% inhibition of mycelial growth (EC_{50}) values of *S. sclerotiorum* ranged from 0.0417 to 0.4732 μg/mL with an average EC_{50} value (mean ± SD) of (0.1968 ± 0.1053) μg/mL. The frequency distribution range curve was narrow and unimodal, and the average EC_{50} value obtained can be used as the baseline sensitivity of *S. sclerotiorum* to bixafen in Henan Province. In order to determine whether bixafen can be mixed with other fungicides with different modes of action, the EC_{50} values of *S. sclerotiorum* to bixafen, carbendazim, fludioxonil, prothioconazole, dimetachlone, metconazole, and their mixtures were determined based on mycelial growth rate method. The results showed that the EC_{50} values for bixafen, carbendazim, fludioxonil, prothioconazole, dimetachlone and metconazole were 0.1256, 0.1122, 0.0229, 0.0651, 0.8057, and 0.0278 μg/mL, respectively. For fungicide mixtures of bixafen and carbendazim, fludioxonil, prothioconazole, dimetachlone or metconazole (1 : 1, 1 : 3, 1 : 5, 3 : 1 and 5 : 1, *V/V*), the synergistic ratio (SR) ranges from 0.54 to 3.57, which indicates additive or synergistic inhibition. These results suggested that bixafen could be used alternately or in combination with carbendazim, fludioxonil, prothioconazole, dimetachlone and metconazole to prevent and delay the development of resistance in *S. sclerotiorum*. These results provide important and scientific information for the control of sclerotinia stem rot and for monitoring the sensitivity of *S. sclerotiorum* to bixafen in Henan Province.

Keywords: bixafen; *Sclerotinia sclerotiorum*; sclerotinia stem rot; baseline sensitivity; mixture

河南省油菜菌核病菌对氯氟联苯吡菌胺及其复配剂的敏感性

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摘 要：由核盘菌 *Sclerotinia sclerotiorum* 引起的菌核病 (sclerotinia stem rot) 是一种破坏性严重的病害，多发于油菜和许多其他阔叶作物，在农业生产中主要采用杀菌剂进行化学防治。氯氟联苯吡菌胺作为一种琥珀酸脱氢酶抑制剂 (SDHIs)，对核盘菌的菌丝生长有较好的抑制作用。为建立河南省核盘菌对氯氟联苯吡菌胺的敏感性基线，采用菌丝生长速率法测定了 2015 年和 2016 年从河南省不同地区采集分离的 119 株核盘菌对氯氟联苯吡菌胺的敏感性。结果表明：氯氟联苯吡菌胺对核盘菌菌丝生长的有效抑制中浓度 (EC_{50}) 值范围为 0.0417~0.4732 μg/mL，平均 EC_{50} 值为 (0.1968 ± 0.1053) μg/mL。EC_{50} 值频率分布范围窄且呈单峰曲线，平均 EC_{50} 值可以作为河南省核盘菌对氯氟联苯吡菌胺的敏感性基线。为明确氯氟联苯吡菌胺是否能与其他不同作用机制杀菌剂复配，采用菌丝生长速率法测定了核盘菌对氯氟联苯吡菌胺、多菌灵、咯菌腈、丙硫菌唑、菌核净、叶菌唑及其混合物的敏感性。结果表明，氯氟联苯吡菌胺、多菌灵、咯菌腈、丙硫菌唑、菌核净和叶菌唑对核盘菌 EC_{50} 值分别为 0.1256、0.1122、0.0229、0.0651、0.8057 和 0.0278 μg/mL。氯氟联苯吡菌胺与多菌灵、咯菌腈、丙硫菌唑、菌核净、叶菌唑 (体积比 1 : 1、1 : 3、1 : 5、3 : 1 和 5 : 1) 复配剂的增效系数 (SR) 值范围为 0.54~3.57，表现为相加或增效作用。综上结果表明，氯氟联苯吡菌胺可以与多菌灵、咯菌腈、丙硫菌唑、菌核净、叶菌唑这 5 种不同类型的杀菌剂通过交替或复配使用，阻止和延缓核盘菌抗药性的进一步发展。研究结果可为油菜菌核病的防治和河南省油菜菌核病菌对氯氟联苯吡菌胺的敏感性监测提供科学依据。

关键词: 氯氟联苯吡菌胺；核盘菌；菌核病；敏感性基线；复配剂

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0 Introduction

Sclerotinia sclerotiorum is one of the most devastating fungal pathogens in the world, which is capable of attacking more than 700 plant species[1]. Sclerotinia stem rot, a destructive fungal disease caused by the fungus *S. sclerotiorum*, presents in oilseed rape and many other broadleaf crops[2]. It can also damage commercial crops such as crucifers, legumes, compositae, etc.[3]. The infection of *S. sclerotiorum* begins when ascospores colonize, bloom and invade the stem[4]. *S. sclerotiorum* infects all aboveground parts of oilseed rape plants, including leaves, stems, flowers and pods. Sclerotinia stem rot is characterized by necrosis of the plant, reduced pod numbers and stunted growth. It may also lead to a decreased yield and quality of oilseed rape[5].

Currently, sclerotinia stem rot is mainly controlled by chemicals[6-7]. The main method to control sclerotinia stem rot is to spray benzimidazole fungicides and the mixtures of fungicides at the flowering stage of oilseed rape[8]. However, with the long-term use of these fungicides, *S. sclerotiorum* caused very obvious resistance to these fungicides such as carbendazim and cyprodinil[9-10]. As the distribution of fungicide-resistant isolates expands each year, the control efficiency of fungicides decreases each year, or even becomes ineffective eventually, leading to control failure[11]. Therefore, there is an urgent need to screen out fungicides with new modes of action and better effects on sclerotinia stem rot. The succinate dehydrogenase inhibitors (SDHIs) belong to the fungicides of pyrazole carboxamides such as penthiopyrad and bixafen. The SDHIs are characterized by a broad spectrum of fungicidal activity and are also effective against agronomically important Ascomycetes[12-13].

Bixafen, discovered by Bayer Crop Science in 2001, has shown high inhibitory activity against many cereal pathogens in field trials[14-15]. Bixafen has a long time of action, various types, high efficiency and strong selectivity. It has excellent internal absorption and conductivity abilities. It has both therapeutic and preventive effects and inhibits the production of spores and the growth of fungal hyphae. As the fungicide is not currently registered on oilseed rape in China, bixafen has not been used in *S. sclerotiorum*. In a previous report, the sensitivity of *S. sclerotiorum* against an SDHI fungicide boscalid was determined. The EC_{50} values of *S. sclerotiorum* to boscalid ranged from 0.0073 to 0.3880 μg/mL, and the mean EC_{50} value was (0.15 ± 0.09) μg/mL. The frequency distribution was unimodal[16]. However, there are few

reports on the application of bixafen to prevent and control *S. sclerotiorum* in Henan Province and other areas in China.

The aim of this study was to investigate the baseline sensitivity of bixafen against *S. sclerotiorum* obtained from oilseed rape fields in Henan, China. To establish the baseline sensitivity of *S. sclerotiorum* to bixafen in Henan Province, the sensitivities of 119 isolates of *S. sclerotiorum* to bixafen were determined based on the mycelial growth rate method. The mixture of fungicides with different modes of action and structural typesis a common and effective method in production. It improves the control efficiency and delays the development of resistance. In order to determine whether bixafen can be mixed with other fungicides, the fungicides with different modes of action, including the phenylpyrrole fungicide fludioxonil, DMI fungicides prothioconazole and metconazole, benzomezole fungicide carbendazim and dicarboxylic fungicide dimetachlone were chosen. The sensitivity of each fungicide mixture to *S. sclerotiorum* was calculated based on the synergistic ratio (SR) values. The results of this study provide important information for the control of *S. sclerotiorum* in Henan Province and the monitoring of the sensitivity of *S. sclerotiorum* to bixafen.

1 Materials and methods

1.1 Isolate collections

In 2015 and 2016, plots with the most serious sclerotium stem rot were selected from Jiaozuo, Luohe, Nanyang, Pingdingshan, Zhengzhou, Zhoukou and other places in Henan Province and the sclerotia were collected randomly at certain distances from the roots and stems of diseased plants. These plants have not been previously exposed to bixafen and other SDHI fungicides. Three to five sclerotia were obtained from each collected plot, then cut into small pieces. After sterilization in a 0.1% sodium hypochlorite disinfection solution for 30 s and dried with sterile filter paper, sclerotia were transferred to potato dextrose agar (PDA: 200 g of potato, 15 g of agar, and 20 g of dextrose per liter of distilled water) plates for 2 days at 25 ℃ in the dark. The purified and retained single mycelium was transferred to a PDA slant, cultivated for 2 days, and stored at 4 ℃[17]. In this study, a total of 119 non-duplicate isolates of *S. sclerotiorum* were collected from these isolates, including Pingdingshan (n = 24), Nanyang (n = 11), Zhengzhou (n = 33), Zhoukou (n = 14), Jiaozuo (n = 15), Luohe (n = 12), and Xuchang (n = 10).

1.2 Fungicides

All fungicides used in this study were technical grade. Bixafen (98% a.i.) was supplied by Shandong Weifang Runfeng Chemical Co., Ltd. (Shandong, China). Carbendazim (98% a.i.) was supplied by Jiangsu Rotam Chemistry Co., Ltd. (Suzhou, China). Fludioxonil (95% a.i.) was supplied by Syngenta Crop Protection Co., Ltd. (Basel, Switzerland). Prothioconazole (97% a.i.) was kindly provided by Shandong Hailier Chemical Co., Ltd. (Shandong, China). Dimetachlone (95% a.i.) was supplied by Jiangxi Heyi Chemical Co., Ltd. (Jiangxi, China). Metconazole (95% a.i.) was provided by the Jiangsu Huifeng Biological Agriculture Co., Ltd. (Yancheng, China). Fungicides were dissolved in the respective solvents, to obtain 10 000 μg/mL stock solutions (Table 1). All stock solutions were stored at 4 ℃.

Table 1 Concentrations of tested fungicides used in the study

Fungicide	Concentrations/(μg/mL)						Solvent
bixafen	0	0.00625	0.025	0.10	0.40	1.60	acetone
carbendazim	0	0.05	0.10	0.20	0.40	0.80	0.1 mol/L HCl
fludioxonil	0	0.05	0.10	0.15	0.20	0.25	methanol
prothioconazole	0	0.00625	0.0125	0.025	0.05	0.10	acetone
dimetachlone	0	0.00625	0.0125	0.025	0.05	0.10	methanol
metconazole	0	0.20	0.40	0.80	1.60	3.20	acetone

1.3 Sensitivity to bixafen

In order to establish the baseline sensitivity to bixafen, the mycelium growth inhibition of 119 isolates of *S. sclerotiorum* was determined[18]. All 119 isolates of *S. sclerotiorum* were transferred from the PDA slants to 9-cm PDA plates and cultured at 25 ℃ in the dark for 2 days. The 5 mm mycelial plugs were cut from the edge of a 2-day-old colony of each *S.*

sclerotiorum isolate and transferred to PDA plates containing bixafen at concentrations of 0.00625, 0.025, 0.10, 0.40 and 1.60 μg/mL and a blank solvent control without fungicide was set. After 2 days of incubation at 25 ℃ in the dark, the diameter of each colony was measured in two perpendicular directions. The average values obtained from the above measurement were used to calculate the mycelial growth rate according to the following formula (1):

$$I/\% = \frac{D_{ck} - D_t}{D_{ck} - 0.5} \times 100 \quad (1)$$

I is the inhibition rate; D_{ck} is the average of the diameter of the control strain, cm; D_t is the average of the diameter of the treated strain, cm. The EC_{50} value was calculated based on the linear relationship between the $\log_{10}$ of each concentration and the probability of inhibition. Based on EC_{50} value, the baseline sensitivity of *S. sclerotiorum* to bixafen was established. Fisher's protected LSD (P = 0.05) was used to compare the mean EC_{50} values[18]. For each group of experiments, three groups of repetitions were set and the experiment was repeated three times.

1.4 Synergistic interaction in mixtures.

The sensitivity of the bixafen mixtures with carbendazim, fludioxonil, dimetachlone, prothioconazole, or metconazole against *S. sclerotiorum* was determined by the degree of inhibition on *S. sclerotiorum* by different concentration gradients and ratios of concentration of the fungicide mixtures above. Isolates of PDS1604 were also carried out according to the above methods. The experiment combined bixafen and carbendazim, fludioxonil, dimetachlone, prothioconazole, metconazole according to (1 : 1, 1 : 3, 3 : 1, 1 : 5, 5 : 1, *V/V*). In this study, 5-mm-diameter plugs were taken from the edge of each colony with a puncher and inoculated on PDA medium containing each concentration (Table 2). After 2 days of incubation at 25 ℃ in the dark, the diameter of each colony was measured in two perpendicular directions. The EC_{50} value was obtained according to the measurement and calculation method described above. The theoretical inhibition concentration of each mixture (EC_{50}(Exp)) was obtained by formula (2)[19]. The SR was calculated according to the formula (3). When SR ⩾ 1.5, the interaction is defined as synergistic. When 1.5 > SR ⩾ 0.5, the interaction is defined as additive. When SR < 0.5, the interaction is defined as antagonistic[20]. The SR value was used to judge the inhibition of each ratio of fungicides to *S. sclerotiorum*. Three groups of replicates were set for each group of experiments and the experiment was repeated twice.

$$EC_{50}(\text{Exp}) = \frac{a+b}{\frac{a}{EC_{50}(A)} + \frac{b}{EC_{50}(B)}} \quad (2)$$

$$SR = \frac{EC_{50}(\text{Exp})}{EC_{50}(\text{Obs})} \quad (3)$$

A, B are combined fungicides; a, b is the proportion of the fungicide in the mixture; EC_{50}(Exp) is the theoretical suppressed medium concentration; EC_{50}(Obs) is the actual measured medium concentration.

1.5 Statistical analysis

DPS software (version 7.05; Zhejiang University, Hangzhou, China) was used to obtain the regression equation of toxicity, the EC_{50} value, and the 95% confidence limit. The bioassay option in DPS was used to determine the EC_{50} value of *S. sclerotiorum*. The resistance ratios were calculated as the EC_{50} value of the *S. sclerotiorum* isolates[21]. The statistical differences between sensitivity of different isolates were analyzed via the least significant difference (LSD) test at P = 0.05. In order to minimize errors, these tests were repeated twice, and the average of both tests was taken as the final result.

2 Results and analysis

2.1 Sensitivity to difenoconazole

The sensitivity of 119 *S. sclerotiorum* isolates to bixafen was determined based on mycelial inhibition. By analyzing the results of these mycelial growth inhibition of isolates, the EC_{50} values of bixafen to the 119 isolates of *S. sclerotiorum* ranged from 0.0417 to 0.4732 μg/mL and the mean EC_{50} value (mean ± SD) was (0.1968 ± 0.1053) μg/mL (Table 3). The frequency distribution range curve was unimodal with a narrow range (Fig.1). The average EC_{50} value obtained can be used as the baseline sensitivity of *S. sclerotiorum* to bixafen in Henan Province.

2.2 Synergistic interaction in mixtures

The results showed that the EC_{50} of the bixafen, carbendazim, fludioxonil, prothioconazole, dimeta-

Table 2 Concentrations of fungicides mixtures used to determine the sensitivity of *S. sclerotiorum*

Mixtures	Volume ratio of fungicide	Fungicide concentration/(μg/mL)					
bixafen : carbendazim	1 : 1	0	0.00625	0.0125	0.025	0.05	0.10
	1 : 3	0	0.00625	0.0125	0.025	0.05	0.10
	1 : 5	0	0.00625	0.0125	0.025	0.05	0.10
	3 : 1	0	0.0125	0.025	0.05	0.10	0.20
	5 : 1	0	0.00625	0.0125	0.025	0.05	0.10
bixafen : fludioxonil	1 : 1	0	0.008	0.016	0.032	0.064	0.128
	1 : 3	0	0.002	0.004	0.008	0.016	0.032
	1 : 5	0	0.004	0.008	0.016	0.032	0.064
	3 : 1	0	0.004	0.008	0.016	0.032	0.064
	5 : 1	0	0.004	0.008	0.016	0.032	0.064
bixafen : prothioconazole	1 : 1	0	0.00625	0.025	0.10	0.40	1.60
	1 : 3	0	0.00625	0.0125	0.025	0.05	0.10
	1 : 5	0	0.00625	0.025	0.10	0.40	1.60
	3 : 1	0	0.00625	0.0125	0.025	0.05	0.10
	5 : 1	0	0.00625	0.025	0.10	0.40	1.60
bixafen : dimetachlone	1 : 1	0	0.00625	0.0125	0.025	0.05	0.10
	1 : 3	0	0.00625	0.025	0.10	0.40	1.60
	1 : 5	0	0.00625	0.025	0.10	0.40	1.60
	3 : 1	0	0.050	0.100	0.20	0.40	0.80
	5 : 1	0	0.00625	0.0125	0.025	0.05	0.10
bixafen : metconazole	1 : 1	0	0.00625	0.025	0.10	0.40	1.60
	1 : 3	0	0.00625	0.025	0.10	0.40	1.60
	1 : 5	0	0.0125	0.025	0.05	0.10	0.20
	3 : 1	0	0.00625	0.025	0.10	0.40	1.60
	5 : 1	0	0.00625	0.025	0.10	0.40	1.60

Table 3 Sensitivity of *S. sclerotiorum* to bixafen in Henan Province in 2015 and 2016

Source	Number of isolates			EC_{50} value range/(μg/mL) [b]	Average EC_{50} value/(μg/mL) [c]
	2015	2016	Total		
Pingdingshan	14	10	24	0.0417-0.4093	0.1802 ± 0.1213a
Zhengzhou	15	18	33	0.0540-0.3407	0.2224 ± 0.0636a
Jiaozuo	15	—	15	0.0878-0.4420	0.2616 ± 0.1117a
Zhoukou	14	—	14	0.0525-0.4262	0.2173 ± 0.1203a
Nanyang	11	—	11	0.0710-0.3580	0.2076 ± 0.1086a
Xuchang	—[a]	10	10	0.1094-0.4732	0.2038 ± 0.1070a
Luohe	—	12	12	0.0626-0.2412	0.1376 ± 0.0540b
Total	69	50	119	0.0417-0.4732	0.1968 ± 0.1053a

[a] Means that no samples were collected. [b] EC_{50}: effective concentration for 50% inhibition of mycelial growth. [c] Means in a column followed by the same lowercase letter were not different according to Fisher's least significant difference (LSD) ($P = 0.05$).

chlone and metconazole against *S. sclerotiorum* was 0.1256, 0.1122, 0.0229, 0.0651, 0.8057, and 0.0278 μg/mL, respectively, indicating that the test fungicides have high inhibitory activity on the growth of *S. sclerotiorum*. The mixture combining bixafen with carbendazim, fludioxonil, sclerotium, prothioconazole, or metconazole according to (1 : 1, 1 : 3, 3 : 1, 1 : 5, 5 : 1, *V/V)* five treatments shows that the SR values against

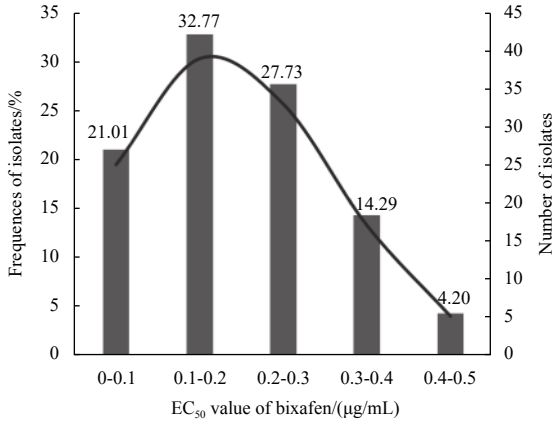

Fig. 1 Distribution of EC_{50} values for sensitivity to bixafen of 119 isolates of *S. sclerotiorum* collected from oilseed rape fields in different regions of Henan Province, China, in 2015 and 2016

the mycelial growth of *S. sclerotiorum* ranged from 0.54 to 3.57 (Table 4). Among them, the highest SR value of 3.57 was obtained by the concentration ratio experiment of bixafen and dimetachlone 1 : 3. These mixtures are synergistic : bixafen and carbendazim (1 : 1, 1 : 3, 3 : 1, 5 : 1, *V/V*), bixafen and fludioxonil (1 : 3, 1 : 5, 3 : 1, 5 : 1, *V/V*), bixafen and prothioconazole (1 : 1, 5 : 1, *V/V*), bixafen and dimetachlone (1 : 1, 1 : 3, 1 : 5, 5 : 1, *V/V*), bixafen and metconazole (1 : 1, *V/V*). Meanwhile, the SR values of all treatments were greater than 0.5. This indicates that the effect of the mixtures of these fungicides is synergistic or additive, which may be due to the influence of the modes of action of each fungicide.

Table 4 Effect of different mixtures on the virulence of *S. sclerotiorum*

Fungicide	Volume ratio of fungicide	Toxic regression equation	Correlation coefficient	EC_{50} (Obs)/ (μg/mL)	EC_{50} (Exp)/ (μg/mL)	Synergic ratio, SR
bixafen	—[a]	$y = 5.8722 + 0.6517x$	0.9723	0.1256	—	—
carbendazim	—	$y = 6.5421 + 1.6234x$	0.9691	0.1122	—	—
fludioxonil	—	$y = 9.4526 + 2.7147x$	0.9229	0.0229	—	—
prothioconazole	—	$y = 6.6898 + 1.4241x$	0.9248	0.0651	—	—
dimetachlone	—	$y = 5.3920 + 4.1768x$	0.9375	0.8057	—	—
metconazole	—	$y = 7.9071 + 1.8682x$	0.9847	0.0278	—	—
bixafen : carbendazim	1 : 1	$y = 6.6709 + 1.1514x$	0.9913	0.0354	0.1185	3.35
	1 : 3	$y = 6.0514 + 0.751x$	0.9948	0.0398	0.1153	2.90
	1 : 5	$y = 6.0220 + 1.016x$	0.9961	0.0961	0.1142	1.19
	3 : 1	$y = 5.8258 + 0.6415x$	0.9865	0.0516	0.1231	2.39
	5 : 1	$y = 6.4809 + 1.261x$	0.9542	0.0669	0.1231	1.84
bixafen : fludioxonil	1 : 1	$y = 6.7106 + 1.3007x$	0.9831	0.0484	0.0387	0.80
	1 : 3	$y = 7.6467 + 1.3213x$	0.9559	0.0099	0.0288	2.91
	1 : 5	$y = 9.1625 + 2.3198x$	0.9903	0.0161	0.0265	1.65
	3 : 1	$y = 6.9226 + 1.142x$	0.9605	0.0207	0.0592	2.86
	5 : 1	$y = 7.0207 + 1.2141x$	0.9875	0.0217	0.0719	3.31
bixafen : prothioconazole	1 : 1	$y = 6.0762 + 0.7213x$	0.9918	0.0332	0.0858	2.58
	1 : 3	$y = 5.7492 + 0.7409x$	0.9798	0.0975	0.0740	0.76
	1 : 5	$y = 6.6200 + 1.2736x$	0.9896	0.0533	0.0708	1.33
	3 : 1	$y = 5.7296 + 0.7102x$	0.9822	0.0939	0.1019	1.09
	5 : 1	$y = 5.8118 + 0.5691x$	0.9941	0.0374	0.1088	2.91
bixafen : dimetachlone	1 : 1	$y = 6.1731 + 1.0274x$	0.9969	0.0721	0.2173	3.01
	1 : 3	$y = 6.0620 + 1.0426x$	0.9596	0.0958	0.3423	3.57
	1 : 5	$y = 5.9075 + 0.9063x$	0.9655	0.1630	0.4235	2.60
	3 : 1	$y = 6.0373 + 1.4324x$	0.9961	0.1868	0.1592	0.85
	5 : 1	$y = 6.3319 + 1.294x$	0.9935	0.0935	0.2173	2.32
bixafen : metconazole	1 : 1	$y = 6.460 + 0.8452x$	0.9677	0.0187	0.0455	2.43
	1 : 3	$y = 5.5723 + 0.6319x$	0.9846	0.1243	0.0668	0.54
	1 : 5	$y = 6.8422 + 1.4368x$	0.9936	0.0522	0.0319	0.61
	3 : 1	$y = 6.3225 + 1.0651x$	0.9545	0.0673	0.0668	0.99
	5 : 1	$y = 5.7527 + 0.6089x$	0.9846	0.0581	0.0792	1.36

[a] Means no data.

3 Conclusion and discussion

The application of a single fungicide induces the resistance of *S. sclerotiorum* in oilseed rape to benzimidazole fungicides, dicarboximide fungicides and SDHI fungicides[22]. At the same time, the resistance of fungicides has a clear trend of stable inheritance, which reduces the control efficiency of these agents on diseases such as sclerotinia stem rot[23-26]. Therefore, the selection of new fungicides with different modes of action as parts of action is currently a key issue[27].

In previous studies, the SDHI fungicides have been used to prevent sclerotinia stem rot. In one study, 120 isolates of *S. sclerotiorum* were collected and isolated from oilseed rape in different areas of Jiangsu Province from 2006 to 2008. The baseline sensitivity of the isolates to boscalid was measured to be 0.028 to 0.398 μg/mL. The average EC_{50} value was (0.17 ± 0.09) μg/mL[28]. In another study from Anhui, Hubei, Jiangsu, and Zhejiang provinces, 161 isolates of *S. sclerotiorum* were collected and isolated from oilseed rape from 2001 to 2008. The baseline sensitivity to boscalid was 0.002-0.391 μg/mL, and the average EC_{50} value was 0.042 μg/mL[29]. In this experiment, there was no significant difference in the concentration of EC_{50} inhibition of 119 isolates of *S. sclerotiorum*, distributed from 0.0417 to 0.4732 μg/mL and the average EC_{50} value of (0.1968 ± 0.1053) μg/mL. Although they were both SDHI fungicides, the geographical origin of the isolates, the number of the tested isolates, and the duration of treatment had a great influence on the sensitivity of *S. sclerotiorum*. In addition, it is reported that SDHI-resistant isolates such as boscalid-resistant isolates have appeared in some areas in China[30].

Bixafen is an SDHI, which acts on the nodes of electron transport and oxidative phosphorylation in fungi, leading to the termination of the tricarboxylic acid cycle, affecting its energy metabolism, hindering the growth and the death of plant-pathogenic fungi, and finally achieving the goal of scientific crop disease control. Fludioxonil belongs to phenylpyrrole fungicide, which is a division protein activated kinase and histidine kinase inhibitor of osmotic signal transduction and inhibits the growth of pathogenic fungal mycelium by inhibiting the transfer related to glucose phosphorylation[31-34]. Prothioconazole is a DMI fungicide, and its mechanism of action is to inhibit the demethylation of lanosterol or 2,4-methylene dihydrolanosterol 14, a sterol precursor in fungi, which affects the biosynthesis of ergosterol and inhibits the growth of pathogenic mycelia[35]. Metconazole, a DMI fungicide, is a C-14 demethylase inhibitor that primarily inhibits ergosterol biosynthesis in plant-pathogenic fungi[36]. Carbendazim, a benzomezole fungicide, is widely used to control many fungal diseases during agricultural production. Its mechanism of action is to inhibit microtubule assembly by binding to β-tubulin or β_2-tubulin genes in pathogenic fungi[37-38]. Dimetachlone is a dicarboxylic fungicide whose mechanism of action is binding to two-component histidine kinase[39]. The results of the single fungicide sensitivity determination study showed that these five test fungicides had good inhibitory effects on the growth of *S. sclerotiorum*. This study showed that when the volume ratio of 1 : 1, 1 : 3, 1 : 5, 3 : 1 and 5 : 1 was used, 15 of the 25 combinations of the mixtures were synergistic. In these ratios, bixafen and dimetachlone (1 : 3, *V/V*) had the best synergistic effect. Other mixtures with different proportions showed additive effects, indicating that the modes of action of each mixture did not affect each other.

This experiment filled a gap in the study of bixafen's sensitivity to *S. sclerotiorum* in Henan Province from 2015 to 2016 because no one had conducted a study of bixafen's sensitivity to *S. sclerotiorum* before. It can also be used to make more scientific guidance for the application of bixafen in Henan Province. The results proved that *S. sclerotiorum* in the seven regions of Henan Province showed sensitivity to bixafen. In order to avoid resistance, bixafen can be used in combination with other fungicides to reduce the frequency of resistance and improve the control efficiency of sclerotinia stem rot.

References:

[1] BORCHERS A, PIELER T. Programming pluripotent precursor cells derived from *Xenopus* embryos to generate specific tissues and organs[J]. Genes, 2010, 1(3): 413-426.

[2] LIU S M, ZHANG Y, JIANG J, et al. Carbendazim resistance and dimethachlone sensitivity of field isolates of *Sclerotinia sclerotiorum* from oilseed rape in Henan Province, China[J]. J Phytopathol, 2018, 166(10): 701-708.

[3] YU Y, DU J, WANG Y B, et al. Survival factor 1 contributes to the oxidative stress response and is required for full virulence of *Sclerotinia sclerotiorum*[J]. Mol Plant Pathol, 2019, 20(7): 895-906.

[4] CHANG H X, SANG H, WANG J, et al. Exploring the genetics of lesion and nodal resistance in pea (*Pisum sativum* L.) to *Sclerotinia*

sclerotiorum using genome-wide association studies and RNA-Seq[J]. Plant Direct, 2018, 2(6): e00064.
[5] LIU S M, FU L Y, CHEN J P, et al. Baseline sensitivity of *Sclerotinia sclerotiorum* to metconazole and the analysis of cross-resistance with carbendazim, dimethachlone, boscalid, fluazinam, and fludioxonil[J]. Phytoparasitica, 2021, 49(1): 123-130.
[6] YANG X X, ZHANG L, XIANG Y J, et al. Author correction: comparative transcriptome analysis of *Sclerotinia sclerotiorum* revealed its response mechanisms to the biological control agent, *Bacillus amyloliquefaciens*[J]. Sci Rep, 2021, 11(1): 5071.
[7] MAO X W, WANG Y F, HOU Y P, et al. Activity of the succinate dehydrogenase inhibitor fungicide penthiopyrad against *Sclerotinia sclerotiorum*[J]. Plant Dis, 2020, 104(10): 2696-2703.
[8] NIE P C, DONG T, XIAO S P, et al. Quantitative determination of thiabendazole in soil extracts by surface-enhanced Raman spectroscopy[J]. Molecules, 2018, 23(8): 1949.
[9] HOU Y P, MAO X W, QU X P, et al. Molecular and biological characterization of *Sclerotinia sclerotiorum* resistant to the anilinopyrimidine fungicide cyprodinil[J]. Pestic Biochem Physiol, 2018, 146: 80-89.
[10] DING L N, LI T, GUO X J, et al. Sclerotinia stem rot resistance in rapeseed: recent progress and future prospects[J]. J Agric Food Chem, 2021, 69(10): 2965-2978.
[11] ZHU Z Q, ZHOU F, LI J L, et al. Carbendazim resistance in field isolates of *Sclerotinia sclerotiorum* in China and its management[J]. Crop Prot, 2016, 81: 115-121.
[12] ISHII H, MIYAMOTO T, USHIO S, et al. Lack of cross-resistance to a novel succinate dehydrogenase inhibitor, fluopyram, in highly boscalid-resistant isolates of *Corynespora cassiicola* and *Podosphaera xanthii*[J]. Pest Manag Sci, 2011, 67(4): 474-482.
[13] AVENOT H F, MICHAILIDES T J. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi[J]. Crop Prot, 2010, 29(7): 643-651.
[14] HUANG X P, LUO J A, SONG Y F, et al. Favorable bioactivity of the SDHI fungicide benzovindiflupyr against *Sclerotinia sclerotiorum* mycelial growth, sclerotial production, and myceliogenic and carpogenic germination of sclerotia[J]. Plant Dis, 2019, 103(7): 1613-1620.
[15] ERNST G, AGERT J, HEINEMANN O, et al. Realistic exposure of the fungicide bixafen in soil and its toxicity and risk to natural earthworm populations after multiyear use in cereal[J]. Integr Environ Assess Manag, 2022, 18(3): 734-747.
[16] LIU S M, FU L Y, HAI F, et al. Sensitivity to boscalid in field isolates of *Sclerotinia sclerotiorum* from rapeseed in Henan Province, China[J]. J Phytopathol, 2018, 166(4): 227-232.
[17] SARMA B K, AMEER BASHA S, SINGH D P, et al. Use of non-conventional chemicals as an alternative approach to protect chickpea (*Cicer arietinum*) from sclerotinia stem rot[J]. Crop Prot, 2007, 26(7): 1042-1048.
[18] THOMULKA K W, ABBAS C G, YOUNG D A, et al. Evaluating median effective concentrations of chemicals with bioluminescent bacteria[J]. Bull Environ Contam Toxicol, 1996, 56(3): 446-452.
[19] GISI U, BINDER H, RIMBACH E. Synergistic interactions of fungicides with different modes of action[J]. Trans Br Mycol Soc, 1985, 85(2): 299-306.
[20] GISI U. Synergistic interaction of fungicides in mixtures[J]. Phytopathology, 1996, 86: 1273-1279.
[21] TANG Q Y, ZHANG C X. Data Processing System (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research[J]. Insect Sci, 2013, 20(2): 254-260.
[22] WANG Y, DUAN Y B, WANG J X, et al. A new point mutation in the iron-sulfur subunit of succinate dehydrogenase confers resistance to boscalid in *Sclerotinia sclerotiorum*[J]. Mol Plant Pathol, 2015, 16(7): 653-661.
[23] DUAN Y B, YANG Y, WANG J X, et al. Development and application of loop-mediated isothermal amplification for detecting the highly benzimidazole-resistant isolates in *Sclerotinia sclerotiorum* [J]. Sci Rep, 2015, 5: 17278.
[24] WANG Y, HOU Y P, CHEN C J, et al. Detection of resistance in *Sclerotinia sclerotiorum* to carbendazim and dimethachlon in Jiangsu Province of China[J]. Australas Plant Pathol, 2014, 43(3): 307-312.
[25] XU D, PAN Y, ZHANG H, et al. Detection and characterization of carbendazim resistance in *Sclerotinia sclerotiorum* isolates from oilseed rape in Anhui Province of China[J]. Genet Mol Res, 2015, 14(4): 16627-16638.
[26] ZHOU F, ZHANG X L, LI J L, et al. Dimethachlon resistance in *Sclerotinia sclerotiorum* in China[J]. Plant Dis, 2014, 98(9): 1221-1226.
[27] DUAN Y B, LIU S M, GE C Y, et al. *In vitro* inhibition of *Sclerotinia sclerotiorum* by mixtures of azoxystrobin, SHAM, and thiram[J]. Pestic Biochem Physiol, 2012, 103(2): 101-107.
[28] WANG J X, MA H X, CHEN Y, et al. Sensitivity of *Sclerotinia sclerotiorum* from oilseed crops to boscalid in Jiangsu Province of China[J]. Crop Prot, 2009, 28(10): 882-886.
[29] LIU X, YIN Y N, YAN L Y, et al. Sensitivity to iprodione and boscalid of *Sclerotinia sclerotiorum* isolates collected from rapeseed in China[J]. Pestic Biochem Physiol, 2009, 95(2): 106-112.
[30] WANG Y, DUAN Y B, ZHOU M G. Molecular and biochemical characterization of boscalid resistance in laboratory mutants of *Sclerotinia sclerotiorum*[J]. Plant Pathol, 2015, 64(1): 101-108.
[31] FURUKAWA K, RANDHAWA A, KAUR H, et al. Fungal fludioxonil sensitivity is diminished by a constitutively active form of the group III histidine kinase[J]. FEBS Lett, 2012, 586(16): 2417-2422.
[32] KUANG J, HOU Y P, WANG J X, et al. Sensitivity of *Sclerotinia sclerotiorum* to fludioxonil: *in vitro* determination of baseline sensitivity and resistance risk[J]. Crop Prot, 2011, 30(7): 876-882.
[33] LEW R R. Turgor and net ion flux responses to activation of the osmotic MAP kinase cascade by fludioxonil in the filamentous fungus *Neurospora crassa*[J]. Fungal Genet Biol, 2010, 47(8): 721-726.
[34] LIU S M, HAI F, JIANG J. Sensitivity to fludioxonil of *Botrytis cinerea* isolates from tomato in Henan Province of China and characterizations of fludioxonil-resistant mutants[J]. J Phytopathol, 2017, 165(2): 98-104.
[35] ZAMANI-NOOR N, Baseline sensitivity and control efficacy of various group of fungicides against *Sclerotinia sclerotiorum* in oilseed rape cultivation[J]. Agronomy, 2021, 11(9): 1758.
[36] DUAN Y B, TAO X A, ZHAO H H, et al. Activity of demethylation inhibitor fungicide metconazole on Chinese *Fusarium graminearum* species complex and its application in carbendazim-resistance management of *Fusarium* head blight in wheat[J]. Plant Dis, 2019, 103(5): 929-937.
[37] LIU S M, LIU J L, FU L Y, et al. Baseline sensitivity of *Fusarium graminearum* from wheat fields in Henan, China, to metconazole and analysis of cross resistance with carbendazim and phenamacril[J]. J Phytopathol, 2020, 168(3): 156-161.
[38] LIU S M, CHE Z P, CHEN G Q. Multiple-fungicide resistance to carbendazim, diethofencarb, procymidone, and pyrimethanil in field isolates of *Botrytis cinerea* from tomato in Henan Province, China[J]. Crop Prot, 2016, 84: 56-61.
[39] ZHOU F, LIANG H J, DI Y L, et al. Stimulatory effects of sublethal doses of dimethachlon on *Sclerotinia sclerotiorum*[J]. Plant Dis, 2014, 98(10): 1364-1370.

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