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Bioactivities of five fungicides against *Rhizoctonia solani*, the causal agent of tobacco sore shin

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Abstract: Tobacco sore shin caused by *Rhizoctonia solani* is one of the most destructive diseases in the seedbed suffered by tobacco in China. This study evaluated the activities of the fungicides azoxystrobin, boscalid, fluazinam, propiconazole, and pyrimethanil against mycelial growth, sclerotia formation and germination of *R. solani*, and also their protective and curative efficacies against tobacco sore shin. The mycelial growth of *R. solani* was more sensitive to fluazinam and azoxystrobin than to propiconazole and boscalid, and least sensitive to pyrimethanil. Azoxystrobin showed stronger inhibition of sclerotia production than propiconazole, fluazinam, pyrimethanil, and boscalid. None of the five fungicides inhibited the sclerotia germination of *R. solani*. In terms of the protective activity of detached tobacco leaves, azoxystrobin and boscalid at 12.5 and 50 mg/L were superior to fluazinam, propiconazole, and pyrimethanil in reducing sore shin. For curative activity, azoxystrobin at 50 and 200 mg/L was superior to the other four fungicides in reducing disease. Therefore, among the five fungicides, azoxystrobin is most suitable for the control of tobacco sore shin.

Keywords: *Rhizoctonia solani*; azoxystrobin; sensitivity; sclerotium production; control efficacy

五种杀菌剂对烟草立枯病菌的生物活性

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摘 要：由立枯病丝核菌引起的烟草立枯病是我国烟草苗床上危害最严重的病害之一。本研究评价了 5 种杀菌剂 (嘧菌酯、啶酰菌胺、氟啶胺、丙环唑和嘧霉胺) 对立枯丝核菌菌丝生长、菌核形成和萌发的影响，以及其对烟草立枯病的防治效果。结果表明：立枯丝核菌菌丝对氟啶胺和嘧菌酯的敏感性高于丙环唑和啶酰菌胺，而对嘧霉胺的敏感性较低；嘧菌酯对菌核形成的抑制作用强于丙环唑、氟啶胺、啶酰菌胺和嘧霉胺；5 种杀菌剂对立枯丝核菌菌核萌发均无抑制作用。在离体烟叶的保护活性方面，12.5 和 50 mg/L 的嘧菌酯和啶酰菌胺对立枯病的保护作用优于氟啶胺、丙环唑和嘧霉胺；在治疗活性方面，50 和 200 mg/L 的嘧菌酯的治疗作用优于其他 4 种杀菌剂。因此，供试的 5 种杀菌剂中嘧菌酯最适合用于烟草立枯病的防治。

关键词: 立枯丝核菌；嘧菌酯；敏感性；菌核形成；防治效果
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Tobacco (*Nicotiana tabacum* L.) is a leafy, annual, solanaceous plant. It is an important economic crop in the world and is commercially grown in China for its leaves[1]. China is the largest single tobacco market in the world, accounting for 40% of global tobacco production and consumption[2]. Tobacco sore shin caused by *Rhizoctonia solani* Kühn, is a destructive disease in the seedbed and frequently occurs during seedling development[3]. All of the tobacco stem bases, leaves, and roots are susceptible. The disease occurs frequently each year in two major tobacco commercial regions: Yunnan and Guizhou provinces. Without chemical control, the loss reaches more than 50%[4]. As a ubiquitous soil-borne pathogen, *R. solani*, infect not only tobacco but also many other hosts including sugar beet[5], rice[6], soybean[7], potato[8], lettuce[9], etc.

Throughout the history of *Rhizoctonia* disease management, many synthetic fungicides have been used to control *R. solani*, including validamycin[10], hymexazol[11], azoxystrobin[12], boscalid[13], propiconazole[14] and fluazinam[15]. It is important to understand the *in vitro* activity of a fungicide against a pathogen at various life cycle stages, especially for the mycelial growth and sclerotium formation and germination of *R. solani*. Additionally, the *in vivo* activity of a chemical against disease is also critical for disease management. In China, four fungicides with different modes of action have been utilized for *Rhizoctonia* disease management on field crops, including azoxystrobin (mitochondrial complex III Qo site inhibitor), boscalid (succinate dehydrogenase inhibitor), fluazinam (uncoupler of oxidative phosphorylation), and propiconazole (sterol biosynthesis inhibitor). However, only older registered fungicides such as hymexazol[16], mancozeb[16], isoprothiolane, and zineb are used for tobacco sore shin management in China. The above fungicides, boscalid, fluazinam, and propiconazole are not yet registered on tobacco in China, and azoxystrobin was only registered on tobacco last year as a compound with difenoconazole. Last but not least, there is no relevant research on the *in vitro* activities of these fungicides against *R. solani* isolated from tobacco in China. Based on previous studies, we found that the sensitivity of the species from different hosts to a fungicide varies. For example, the EC_{50} values of difenoconazole against *R. solani* isolated from tobacco and wheat were (0.55 ± 0.53) μg/mL and (0.165 ± 0.101) μg/mL, respectively[16-17]. The EC_{50} values of carbendazim against *R. solani* isolated from tobacco, cotton, and sesame were 0.06, 0.606, and 1.064 μg/mL, respectively[16, 18-19]. Therefore, the objectives of this study were to (i) evaluate the *in vitro* activities of the fungicides azoxystrobin, boscalid, fluazinam, and propiconazole during different life stages (mycelial growth, sclerotium formation, and sclerotium germination) of *R. solani*; (ii) assess the *in vivo* activities of those chemicals against tobacco sore shin. The fungicide pyrimethanil, which is normally sprayed on the seedbed to control tobacco gray mold, was selected as a control. This study would provide useful information for the management of tobacco sore shin in China.

1 Materials and methods

1.1 Pathogen, medium, plant, and fungicide preparation

During the epidemic season of tobacco sore shin in 2017, *R. solani* isolates (**1-1**, **2-2**, **3-2**, and **4-2**) were collected from infected tobacco stems in two commercial greenhouses where no fungicides were used in Guizhou Province of China. AEA medium ($NaNO_3$ 6 g/L, yeast powder 5 g/L, KH_2PO_4 1.5 g/L, KCl 0.5 g/L, $MgSO_4$ 0.25 g/L, glycerol 20 mL/L, and agar 16.0 g/L)[1] was used throughout the experiment. For long-term storage, agar plugs (5 mm in diameter) of *R. solani* from the leading edge of individual colonies were transferred to AEA slants and maintained at 4 ℃. A susceptible tobacco *cv*. Yunyan 85 was utilized as the host of tobacco sore shin. For *in vivo* bioassay, tobacco plants were cultivated from seeds in a 2-liter pot in the greenhouse under natural conditions. When the eight leaves were fully developed eight weeks later, the first 3-4 leaves were selected for testing.

Technical grade azoxystrobin (99.9% active ingredient (a.i.)), boscalid (99.9% a.i.), fluazinam (99.9% a.i.), propiconazole (99.9% a.i.), and pyrimethanil (96% a.i.) used in this study were all dissolved in methanol to prepare 10 000 mg/L stock solutions and stored at 4 ℃ in the dark before use. The methanol concentration does not exceed 0.5% in the testing solutions. It has been demonstrated 0.5%

or less methanol did not affect the mycelial growth, sclerotium production and germination of *R. solani* (data not shown). The control always contained the same methanol concentration as the test samples. Commercial fungicides used in *in vivo* study were azoxystrobin (Amistar, 250 g/L a.i., SC, Syngenta, China), boscalid (Endura, 50% a.i., WDG, BASF, China), fluazinam (Frown Cide, 500 g/L a.i., SC, Ishihara Sangyo Kaisha Ltd., China), propiconazole (Tilt, 250 g/L a.i., EC, Syngenta, China), and pyrimethanil (Scala, 400 g/L a.i., SC, Bayer, China).

1.2 Inhibition of mycelial growth

Isolates of *R. solani* were incubated on AEA plates for 2 days. Individual agar plugs (5 mm in diameter) were removed from the edge of an actively growing colony and incubated face down on the center of a Petri dish (9 cm in diameter), which contained various concentrations of each test fungicide. The final test concentrations were 0, 0.31, 0.62, 1.25, 2.5, 5, and 10 mg/L for azoxystrobin, boscalid, and fluazinam; 0, 0.13, 0.25, 0.5, 1, and 2 mg/L for propiconazole; and 0, 0.016, 0.031, 0.062, 0.125 and 0.25 mg/L for pyrimethanil. There were four biological replicates for each concentration of fungicide, and the entire experiment was conducted twice. After inoculation with the agar plug of *R. solani*, plates were incubated at 25 ℃ in dark. After three days of incubation, the colony diameter was determined as the mean of two measurements perpendicular to each other, and the initial diameter of the mycelial plug was subtracted[1].

1.3 Inhibition of sclerotium production

Agar plugs of each isolate of *R. solani* were prepared as mentioned above. The inhibition of sclerotia production by each fungicide was conducted on AEA plates using the same method as that of mycelial growth. The final test concentrations were 0, 0.1, 1, 10, and 100 mg/L for azoxystrobin; 0, 0.625, 1.25, 2.5, 5, and 10 mg/L for boscalid; 0, 0.0313, 0.0625, 0.125, 0.25 and 0.5 mg/L for fluazinam; 0, 0.313, 0.625, 1.25, 2.5 and 5 mg/L for propiconazole; and 0, 4, 8, 16 and 32 mg/L for pyrimethanil. For each fungicide at each concentration, four biological replicates were performed, and the entire experiment was conducted twice. After inoculation, plates were incubated at 25 ℃ in dark for sclerotium production. Thirty days later, sclerotia produced on each plate were harvested with sterile tweezers and weighed.

1.4 Inhibition of sclerotium germination

Isolates of *R. solani* were incubated on AEA plates at 25 ℃ for 30 days. The sclerotia produced on the plates were harvested on a sterile bench. For sclerotium germination inhibition test, twelve sclerotia were soaked in different concentrations of fungicide for 30 s, then quickly removed and placed on absorbent paper, and incubated on AEA medium after the fungicide dries up[20]. The final test concentrations were 0, 200, 500 mg/L for azoxystrobin; 0, 50, 100 mg/L for boscalid; 0, 10, 40 mg/L for fluazinam; 0, 50, 100 mg/L for propiconazole; and 0, 500 1000 mg/L for pyrimethanil. For each fungicide at each concentration, four biological replicates were performed, and the entire experiment was conducted twice. After inoculation, plates were incubated at 25 ℃ in dark for four days. The number of germinated sclerotia on each plate was counted and the germination ratio of sclerotia for each concentration of each fungicide was calculated. Sclerotium germination is estimated with the appearance of mycelium around the sclerotium[21].

1.5 Protective and curative activities against tobacco sore shin on detached leaves

For protective and curative activities of azoxystrobin, boscalid, fluazinam, propiconazole, and pyrimethanil against tobacco sore shin, the first 3-4 detached tobacco leaves were used. They were cleaned with distilled water, air-dried, and sprayed with fungicide suspension. The final test concentrations were 0, 3.13, 12.5, 50, 200, and 800 mg/L for all fungicides. To detect the protective activity, fungicides were sprayed on detached leaves 24 hours prior to inoculation. To determine the curative activity, fungicides were sprayed on detached leaves 24 hours after inoculation. Inoculation on detached leaves was conducted with mycelial plugs of *R. solani*. A superficial wound (3 mm) was made on each leaf with a sterile needle. Afterward, a mycelial plug (5 mm) was taken from the edge of a 3-day-old colony on AEA medium and placed inverted on the leaf wound. Eight biological replicates were conducted for each concentration of each fungicide, and the entire experiment was conducted twice. After inoculation, detached leaves were incubated at 25 ℃ for 24 h in dark, and then kept in a climate chamber at 25 ℃, relative humidity > 90%, 100-120 $\mu Em^{-2}\ s^{-1}$, and 14 h light per day for

disease development. Seven days after inoculation, the diameters of each diseased area on the leaves were measured. Then all diameters were combined for the average diameter calculation.

1.6 Data analysis

Data from repeated experiments were combined for analysis to decrease the variances. The SIGMASTAT Statistical Software Package (SPSS Science, Ver. 11) was used for data analysis. The concentration of each fungicide causing 50% (EC_{50}) and 90% (EC_{90}) reduction in mycelial growth, sclerotium production and germination were estimated from the fitted regression line of the log-transformed percentage inhibition plotted against the log-transformed fungicide concentration[22].

2 Results

2.1 Inhibition of mycelial growth

Five tested fungicides exhibited different activities against the mycelial growth of *R. solani*. Fluazinam and azoxystrobin showed the highest inhibition with average EC_{50} values of 0.21 and 0.22 mg/L and average EC_{90} values of 1.24 and 73.07 mg/L, respectively. Mycelial growth was less affected by propiconazole and boscalid, with EC_{50} values of 1.43 and 2.37 mg/L and EC_{90} values of 16.45 and 18.01 mg/L, respectively. In comparison, pyrimethanil had the poorest inhibition with EC_{50} and EC_{90} values of 20.26 and >200 mg/L, respectively (Table 1).

Table 1 Inhibitory effects of five fungicides against the mycelial growth of *R. solani* from tobacco

Fungicide	Isolate	Toxicity regression equation	Correlation coefficient (r)	EC_{50}/(mg/L)	EC_{90}/(mg/L)	Average value of EC_{50}/(mg/L)	Average value of EC_{90}/(mg/L)
fluazinam	1-1	$y = 1.5866x + 6.0185$	0.9767	0.23	1.47	0.21 ± 0.02 b	1.24 ± 0.21 b
	2-2	$y = 1.7362x + 6.3328$	0.9664	0.17	0.93		
	3-2	$y = 1.7288x + 6.1888$	0.9646	0.21	1.13		
	4-2	$y = 1.5569x + 6.0432$	0.9680	0.21	1.42		
azoxystrobin	1-1	$y = 0.4280x + 5.5625$	0.8799	0.05	47.88	0.22 ± 0.08 b	73.07 ± 33.89 b
	2-2	$y = 0.4894x + 5.2934$	0.8874	0.25	104.52		
	3-2	$y = 0.6272x + 5.3509$	0.8634	0.28	30.47		
	4-2	$y = 0.4976x + 5.2670$	0.8564	0.29	109.39		
propiconazole	1-1	$y = 1.0526x + 4.7067$	0.9981	1.90	31.35	1.43 ± 0.25 b	16.45 ± 7.45 b
	2-2	$y = 1.3645x + 4.8999$	0.9921	1.18	10.29		
	3-2	$y = 1.2461x + 4.9040$	0.9908	1.19	12.75		
	4-2	$y = 1.4307x + 4.7691$	0.9977	1.45	11.41		
boscalid	1-1	$y = 1.6667x + 4.7136$	0.9621	1.49	8.73	2.37 ± 0.44 b	18.01 ± 6.66 b
	2-2	$y = 0.9312x + 4.5564$	0.9818	2.99	21.23		
	3-2	$y = 1.2317x + 4.4972$	0.9500	2.56	28.10		
	4-2	$y = 1.6905x + 4.3459$	0.9367	2.44	13.97		
pyrimethanil	1-1	$y = 1.0614x + 3.5380$	0.9942	23.85	384.49	20.26 ± 1.86 a	220.88 ± 81.81 a
	2-2	$y = 1.3498x + 3.2382$	0.9841	20.19	179.76		
	3-2	$y = 1.2823x + 3.3945$	0.9902	17.87	178.44		
	4-2	$y = 1.4778x + 3.1063$	0.9871	19.12	140.82		

Note: Data followed by the same letters in columns are not significantly different (P<0.05) according to Duncan's new multiple range method.

2.2 Inhibition of sclerotium production

Five tested fungicides exhibited different activities against the sclerotium production of *R. solani*. The highest inhibition was recorded by azoxystrobin, which showed 100% inhibition at all test concentrations ranging from 0.1 to 100 mg/L. Boscalid exhibited 18.13% inhibition at the highest concentration of 10 mg/L, while below 5 mg/L it showed promotion of sclerotium production. The inhibition rates of fluazinam, propiconazole, and pyrimethanil at their highest dosages were 32.52%, 69.95%, and 63.42%, respectively, after 30 days of incubation of *R. solani* on AEA plates (Table 2).

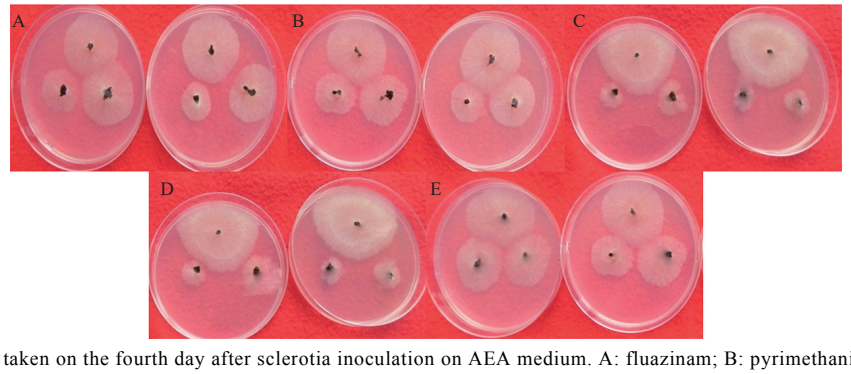
2.3 Inhibition of sclerotium germination

After four days of incubation, all sclerotia of *R. solani* soaked in the fungicide germinated on AEA plates.

Table 2 Inhibitory effects of five fungicides against sclerotium production of *R. solani* from tobacco

Fungicide	Concentration/(mg/L)	Inhibition rate/%				
		Isolate **1-1**	Isolate **1-2**	Isolate **3-2**	Isolate **4-2**	Average
azoxystrobin	0.1	100	100	100	100	100
	1	100	100	100	100	100
	10	100	100	100	100	100
	100	100	100	100	100	100
boscalid	0.625	−23.85	−11.95	−11.11	−81.82	−32.18 ± 24.82
	1.25	16.15	−23.27	−17.04	−71.21	−23.84 ± 23.68
	2.5	21.54	−32.08	−39.26	−53.03	−25.71 ± 23.62
	5	50.77	−5.66	−42.22	−29.55	−6.67 ± 29.22
	10	59.62	9.43	14.07	−10.61	18.13 ± 20.75
fluazinam	0.0313	1.82	25.64	14.81	47.92	22.55 ± 14.23
	0.062 5	16.97	19.23	−6.17	51.39	20.36 ± 15.52
	0.125	4.85	41.03	14.81	61.88	30.64 ± 20.81
	0.25	16.97	16.67	32.10	50.08	28.96 ± 12.14
	0.5	33.33	24.36	20.99	51.39	32.52 ± 9.84
propiconazole	0.313	20.74	41.38	53.33	48.00	40.86 ± 10.06
	0.625	39.26	39.08	46.67	56.00	45.25 ± 6.08
	1.25	24.44	70.69	75.00	61.33	57.87 ± 16.71
	2.5	22.22	77.59	62.50	80.00	60.58 ± 19.18
	5	44.44	75.86	77.50	82.00	69.95 ± 12.76
pyrimethanil	4	6.50	28.40	54.90	26.67	29.12 ± 12.89
	8	13.01	12.35	39.22	43.33	26.98 ± 14.30
	16	31.71	62.96	33.33	72.22	50.06 ± 17.54
	32	36.59	71.60	58.82	86.67	63.42 ± 15.72

None of azoxystrobin, boscalid, fluazinam, propiconazole, and pyrimethanil showed inhibition on sclerotium germination (Fig. 1). However, the treatments with fungicide soaking inhibited mycelial growth of the germinated sclerotia, and propiconazole and azoxystrobin exhibited the most obvious inhibition (Fig. 1C and 1D).



Note: The picture is taken on the fourth day after sclerotia inoculation on AEA medium. A: fluazinam; B: pyrimethanil; C: propiconazole; D: azoxystrobin; E: boscalid. Three sclerotia were on the same plate, the top one was soaked with sterile water without fungicide, the bottom left one was soaked with a high dose of fungicide and the bottom right one was soaked with a low dose of fungicide.

Fig. 1 Inhibitory effects of five fungicides against sclerotium germination of *R. solani* isolated from tobacco

2.4 Protective and curative activities against tobacco sore shin on detached leaves

Five tested fungicides presented different inhibitory efficacy against tobacco sore shin on detached leaves. With the enhancement of fungicide concentration for both protective and curative tests, the efficacy increased greatly for each fungicide. When protective tests were conducted at 200 mg/L, all five fungicides

exhibited nearly 100% efficacy; in the curative tests, azoxystrobin, boscalid, fluazinam, and propiconazole all showed more than 90% efficacy, poorest efficacy was found by pyrimethanil at 83.33%. When protective tests were conducted at 12.5 mg/L, boscalid showed the highest efficacy (100%), followed by azoxystrobin (92.31%), fluazinam and propiconazole showed the poorer effect, and pyrimethanil showed the poorest control efficacy (64.62%); while in the curative tests, fluazinam had the highest efficacy (71.18%), other four fungicides showed poorer efficacy (< 70%) (Table 3).

Table 3 Protective and curative efficacies of five fungicides against sore shin on tobacco detached leaves

Fungicide	Action type	Control efficacy/%				
		3.13 mg/L	12.5 mg/L	50 mg/L	200 mg/L	800 mg/L
azoxystrobin	Protective	58.93 a	92.31 b	98.23 a	100 a	100 a
	Curative	37.98 c	68.30 d	96.97 ab	99.14 a	100 a
boscalid	Protective	53.97 b	100 a	100 a	100 a	100 a
	Curative	48.88 b	62.81 d	64.78 d	93.30 b	100 a
fluazinam	Protective	62.68 a	77.49 c	85.56 b	100 a	100 a
	Curative	52.12 b	71.18 c	79.68 c	96.54 b	100 a
propiconazole	Protective	60.13 a	78.40 c	88.64 b	99.33 a	100 a
	Curative	39.75 c	56.87 e	64.06 d	94.71 b	100 a
pyrimethanil	Protective	3.85 d	64.62 d	78.46 c	100 a	100 a
	Curative	3.11 d	60 d	77.78 c	83.33 c	93.33 b

Note: Data followed by the same letters in columns are not significantly different ($P < 0.05$) according to Duncan's new multiple range method.

3 Discussion

Tobacco sore shin is a notorious disease that happened frequently in the seedling bed in Southwest China[16]. It is necessary to establish a new efficient disease control strategy for this region. Several chemicals have shown high activities against *R. solani* at different life cycle stages or perform well in controlling tobacco sore shin. Our study has presented the strengths and weaknesses of five fungicides at various development stages of *R. solani* and their efficacies in controlling sore shin on tobacco.

Among these chemicals, the mycelial growth of *R. solani* was more sensitive to fluazinam and azoxystrobin than to propiconazole and boscalid, and least sensitive to pyrimethanil. Similar findings have also been reported for these fungicides against other *Rhizoctonia* diseases, including rice sheath blight[13,23-24], sesame damping-off[25], and sugar beet damping-off[26]. Whether such inhibitory effects on mycelial growth are also present within treated tobacco leaves depends on the systematicity and stability of the fungicides in the plant. Azoxystrobin, boscalid, and propiconazole all have good systemic properties and have been reported to have good inhibitory effects on mycelial growth within many treated plant tissue[27-29]. In contrast, fluazinam is a non-systemic compound and pyrimethanil has local-systemic activity. Therefore, they were reported to have limited inhibition of mycelial growth within plant tissue[30-31]. In our study, all five fungicides showed both protective and curative efficacies against tobacco sore shin *in vivo* on detached leaves. Three systemic fungicides (azoxystrobin, boscalid, and propiconazole) presented better protective efficacy at 50 mg/L, while fluazinam with the highest *in vitro* activity against mycelial growth of *R. solani* showed poorer efficacy than azoxystrobin, which might be related to the non-systemic properties of fluazinam. Pyrimethanil registered for the control of tobacco gray mold presented poor activity against tobacco sore shin in this study. During tobacco seedling development in Southwest China, sore shin and gray mold usually occur simultaneously, fungicides with activities against both *Botrytis cinerea* and *R. solani,* such as azoxystrobin and boscalid, could be used to control both diseases.

The fungus *R. solani* complex is a taxonomic entity with multinucleate cells, lacking conidia but producing sclerotia[32]. Sclerotia of *R. solani* are compact bodies of aggregated melanized hyphae, which help the pathogen survive under many unfavorable conditions. Tissue-borne sclerotia are regarded

as the most important source of inoculum[33], especially in the continuous use of tobacco seedbeds. Many researches have focused on the formation and germination of *R. solani* sclerotia, but complete eradication of tissue-borne sclerotia has never been achieved[34-36]. In our study, none of the five chemicals presented inhibition activity against the germination of sclerotium. Similar findings are also reported by other research conducted with other chemicals and biological degradation[33].

Fungicides tested in this study differ in their mode of action at the biochemical level. Azoxystrobin belongs to the QoI fungicide that inhibits mitochondrial respiration by blocking electron transfer from the cytochrome bc1 complex[22]. It completely inhibited the sclerotia formation of *R. solani* in the range of 0.1 to 100 mg/L. Boscalid belongs to the inhibitor of succinate dehydrogenase on mitochondrial electron transport chain complex II[37]. It enhanced the sclerotia formation of *R. solani* at dosages from 0.625 to 5 mg/L. The reason for this result is not yet clear to us and this will be explored in future studies. Fluazinam is an uncoupler of oxidative phosphorylation and inhibits ATP production in fungi[38]. Propiconazole is a demethylation inhibitor that acts on the fungal lanosterol-14α-demethylase in sterol biosynthesis[39]. Pyrimethanil inhibits methionine biosynthesis in fungi[40]. Fewer sclerotia of *R. solani* were produced when higher concentrations of these three fungicides were applied. The reason for this may be that melanized hyphae aggregated less at higher chemical concentrations. Previous researches reported that sclerotia formation and differentiation of *R. solani* were triggered by their thiol-redox state or oxidative stress. The fungicides tested in this study may affect the thiol-redox state or oxidative stress of *R. solani,* especially for the QoI fungicide azoxystrobin.

Of the five fungicides tested in this study, azoxystrobin showed good control of *R. solani*. To our knowledge, azoxystrobin likely has not yet been registered for the control of tobacco sore shin in major tobacco-producing countries such as China, Brazil, India, the USA, Indonesia, Zimbabwe, etc. Instead, it is mainly used for the control of *R. solani* in sugar beet, rice, and soybean. Therefore, if we further refine the efficacy trials and safety trials of azoxystrobin on live plants in the field, this will lay the foundation for the registration of azoxystrobin on tobacco.

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