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Development and Application of Seed Coating Agent for the Control of Major Soil-Borne Diseases Infecting Wheat

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Abstract: In order to reduce the usage amount of pesticide fertilizers and protect the natural environment, seed coating agents are receiving increased wide concern. In this study, the active constituent (pesticide) and inactive components (surfactants and film former) of the seed coating agents were screened and optimized by the wet sand processing superfine grinding method. The fungal inhibition test of pesticides showed that thifluzamide, fludioxonil, pyraclostrobin, and difenoconazole have an obvious fungal inhibitory effect on wheat sharp eyespot, take-all, and root rot. LAE-9 and polyacrylamide + carboxymethyl cellulose (CMC) is recommended for the safe surfactant and film former, respectively, based on the seed germination test. Moreover, 6% difenoconazole · fludioxonil flowable concentrate for seed coating (FSC) stimulates the seedling growth of wheat, advances the growth of root, and improves biomass in the field trial, meanwhile, the control efficiency reached above 80%. Thus, we suggested it can be used as an effective seed coating agent for the control of soil-borne diseases in wheat. The seed coating agent has the characteristics of disease prevention, increasing crop yield, and safety of environment, which is of significance in practical application.

Keywords: seed coating agents; fungicide; wheat; soil-borne diseases; seed germination

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely grown small-grain cereal crops around the world playing important role in the agricultural economy [1]. 736.8 million tonnes of wheat were produced globally in 2015/2016 that worth approximately US 145 billion dollars [2]. With the increasing world population, the demand for crop products, combined with food security and balanced nutrition, is rapidly increasing. The demand for wheat is expected to increase by 60% by 2050 [3]. Crop yields and their associated economic losses are major global concerns in modern agriculture.

In wheat-producing regions, more than 30 kinds of pests and diseases occur on wheat every year, of which soil-borne diseases are one of the greatest constraints to wheat productivity [4]. Wheat sharp eyespot caused by *Rhizoctonia cerealis* van der Hoeven [5], root rot by *Bipolaris sorokiniana* [6], and wheat take-all by *Gaeumammomyces graminis* var. tritici [7] are the important typical soil-borne diseases threatening wheat production worldwide. Moderate and severe soil-borne diseases can result in grain yield losses (an annual loss of ~20%) and poor grain quality [8]. Therefore, it is essential to effectively control soil-borne diseases. However, due to the broad host ranges and difficulties of targeting the pathogen population in the soil, typical soil-borne diseases are hard to control with chemistry and the time-consuming breeding for disease resistance [3].

As one of the pre-sowing seed treatments, seed coating has been widely applied for many crops around the world. The seed coating agents are generally composed of active constituent (pesticide and plant growth regulator) and inactive component, including film-forming agent, suspension concentrate, and pigment. Seed coating with pesticides has been shown to promote plant growth, increase wheat yield, and prevent diseases infestation. For example, during the later stages of development, Mnasri et al. (2017) showed that coating seed is able to protect the crop against seed-and soil-borne pathogens and offer the advantage to active plant defense responses [9]. Previous studies also reported that coating seed enhances the plant stress tolerance in the stage of seed germination and promotes seedling growth under chilling and saline stress [10,11]. However, traditional seed coating agents are usually harmful to people, animals, and the environment because of the toxic pesticides, such as the imidacloprid and carbofuran [12]. In addition, the effectiveness of seed coating agents significantly affected by active constituent, coating ratio, and method. Therefore, the preparation of a novel effective and safe seed coating agent has become a significant research topic.

To meet this demand and protect wheat yield potential, we evaluated the toxicity and plant growth effects of different pesticide and adjuvant on wheat seed. Then, an optimal formulation was studied to develop a high-efficiency and safe seed coating agent for the control of soil-borne diseases. For the seed coating agents, the effectiveness on the disease infections and growth index was further validated in the field.

2. Materials and Methods

2.1. Formatting of Mathematical Components

The seeds of wheat used in this study were free from chemicals and were stored at room temperature. The wheat cultivar used in this study was Jimai 22, one of the most popular winter wheat cultivars in China. It is moderately resistant to comprehensive disease.

The *Rhizoctonia cerealis* van der Hoeven, *Bipolaris sorokiniana*, and *Gaeumammomyces graminis* var. tritici strains were isolated from infected wheat in Soochow (China). The fungal strains were cultivated by potato dextrose agar (PDA) mediums at 25 °C in the dark and stored on PDA slants at 4 °C before use.

2.2. Chemicals

Pesticides, including triadimefon, tebuconazole, fludioxonil, tetraconazole, epoxiconazole, flusilazole, boscalid, and thifluzamide, were purchased from *ANPEL* Laboratory Technologies (Shanghai) Inc. (Shanghai, China), and all had >96% purity. Agricultural additives obtained from Haian Petrochemical Factory (Nantong, China), and the details are as shown in Table S1. All solvents and other chemicals used in the study were of analytical grade.

2.3. Laboratory Toxicity Evaluation

A mycelial radial growth inhibition method was used to determine the toxicity of tested pesticides [13]. Appropriate volumes of the stock solutions of pesticides in acetone were added to the PDA medium immediately before it was poured into culture dishes. Each concentration was tested in quintuplicate. Then, a fungal plug (0.6 cm in diameter) from a 7-day-old PDA culture was placed in the center of the 9-cm-diameter dish containing PDA medium. For the ability of the pesticides to inhibit the fungal growth, the radius of the fungal colony in front of the bacterium after six days of incubation at 28 °C was measured. Media treated with acetone were used as a negative control, water as a blank control. Moreover, growth inhibition was calculated as described in Reference [14].

2.4. Preparation of the Seed Coating Agent

The seed coating agent was prepared by the wet sand processing superfine grinding method [15]. The optimal formula for the seed-coating agent was prepared through an orthogonal test, the procedure conditions were as follows: pesticides with and without surfactants were prepared by dispersing the biopolymers (2% w/v) in deionized water. The water and surfactant ratios were selected on the basis of previous studies. A rotor–stator homogenizer to make the pesticide active ingredient formed a stable dispersion system. Then, other additives (including the film-forming auxiliaries, plant growth regulators, etc.) were added to the aqueous solution according to the certain ratio, and the solution was continuously stirred at 25 °C for 4–5 h until completely dissolved. Meanwhile, the preparation of a novel seed coating agent was completed, and the stability was measured by the recommended Collaborative International Pesticides Analytical Council (CIPAC) method, which are all well fulfilled the demands of pesticide preparation.

2.5. Seed Film Coating Treatment and Germination Test

The wheat seeds were surface sterilized with 2% sodium hypochlorite for 5 min, and then washed thoroughly with autoclaved distilled water. The wheat seeds were film-coated by stirring and the coating agent was applied at a certain coating ratio at 30 °C for 24 h. After coating, the seeds were air-dried for 30 min at room temperature. Then, 25 disinfected seeds were sown in per germination box and were watered daily with 0.5 Hoagland's nutrient solution. The seeds corresponding to each treatment were germinated at 10 or 25 °C for 14 days in darkness, using a growth chamber previously sterilized by ultraviolet radiation at 254 nm for 16 h. Germination counts were recorded at 4 and 7 days under 10 and 25 °C, respectively, after treatment, and 7 and 14 days under 10 and 25 °C for germination potentiality investigation. Germination was calculated by the international rules for seed testing (ISTA, 2006), and each treatment replicated three times.

2.6. Field Trial for the Seed Coating Agent

A field trial was conducted from 2018 to 2019 at two sites: Longkang and Yingshang in Anhui province (China). In this study, the experiments were designed as a randomized block design with each treatment consisting of a 2.0 m × 3.0 m plot separated from each other by a row 20 cm wide. All test seeds were film-coated by hand and the coating agent was applied at a rate of 1 mL per 100 g of seeds. Then, approximately three hundred seeds were planted for each plot with three replications. A random sample of 100 seedlings was selected to determine the seedling quality before transplanting (30 days). After heading, 100 plants were randomly selected to determine the control effect for the soil-borne diseases infection.

2.7. Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). The data were statistically analyzed separately for each experiment using one-way ANOVA on the SPSS software (ver.22.0; SPSS Company, Chicago, IL, USA) [16]. Statistical significance was defined as p values of 0.05. Figures in the study were drawn using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, USA).

3. Results and Discussion

3.1. Laboratory Selecting of Fungicides for Three Soil-Borne Diseases Control in Wheat

Table 1 presents the detailed results on the antifungal efficiency of the different fungicides. In this study, significant toxicity differences were observed between the different fungicides on the three soil-borne diseases. Comparison of results showed that thifluzamide (half-maximal effective concentration, EC_{50}; 0.0189 mg/kg), flusilazole (EC_{50} = 0.0569 mg/kg), and fludioxonil (EC_{50} = 0.0101 mg/kg) treatment provided the best inhibitory control of wheat sharp eyespot, take-all, and root rot, respectively. Additionally, we also found that pyraclostrobin and difenoconazole showed an inhibitory activity among fungal strains which ranged between 0.1344–6.8654 mg/L of EC_{50}. We also noted that the toxicity of single pesticide on soil-borne diseases obviously lower than that of compound pesticide, as described by previous studies [17,18]. Different pesticides can interact synergistically on growth inhibition of plant diseases. Findings from this and other studies, therefore, suggested that compound fungicides had a better disease prevention effect on the three soil-borne diseases.

Table 1. Half-maximal effective concentration (EC50) of 8 fungicides against the three soil-borne diseases control in wheat.

Pesticides	Sharp Eyespot		Take-All		Root Rot	
	EC_{50} (mg/L)	95% FL (mg/L)	EC_{50} (mg/L)	95% FL (mg/L)	EC_{50} (mg/L)	95% FL (mg/L)
Triadimefon	0.3580 c	0.1174–0.6693	0.4522 c	0.1171–0.7433	3.2551 cd	1.7432–5.6411
Tebuconazole	0.0398 f	0.0104–0.0661	0.1749 d	0.0976–0.3451	0.0415 g	0.0221–0.0743
Fludioxonil	0.0651 e	0.0247–0.0932	11.4912 b	7.4532–17.4431	0.0101 h	0.0071–0.0312
Tetraconazole	0.5651 b	0.2140–0.9871	0.0629 e	0.0245–0.0932	1.8586 e	0.7433–2.6547
Epoxiconazole	0.1605 d	0.0562–0.4032	0.0656 e	0.0310–0.0922	0.0422 g	0.0132–0.0663
Flusilazole	0.5307 bc	0.1774–0.9663	0.0569 e	0.0231–0.0840	0.1106 f	0.0654–0.3043
Boscalid	0.1431 d	0.0743–0.4176	-	-	411.9135 a	273.1792–604.1447
Thifluzamide	0.0189 d	0.0092–0.0336	27.8623 a	17.3366–38.2431	1346.796 b	943.8422–1744.3263
Pyraclostrobin	1.3692 a	0.0741–2.8320	0.1931 d	0.0112–0.4663	5.8622 c	3.3370–8.9132
Difenoconazole	0.1344 d	0.0782–0.3024	6.8654 c	2.7173–11.3359	2.1962 de	1.4241–4.4203

The EC_{50} values were subjected using the Probit analysis. FL, Fiducial limits; Means within a column followed by the different letters (e.g, a, b, and c) are significantly different ($p < 0.05$).

3.2. *Effect Analysis of Different Additives on Seed Germination*

The effect of different surfactants and film composition on seed germination were carried out to screen the effective adjuvants. Based on the indicators (germination effect), we obtained an optimum formula by using the wet sand processing superfine grinding method.

Surfactants are included in the coating formulation to improve its distribution onto the seed surface, usually between 6% and 12% of the formulation by weight [11]. In view of the potential toxicity of surfactants [19], eleven frequently used nonionic surfactants were evaluated. The results showed that surfactant had a significant effect on the germination of wheat seeds (Figure 1). By comparison, SG-6 and A-115 induced the largest negative effect, followed by SG-6, A-115, EL-60, T-20, JFC, and JFC-E, and the inhibition in weight and germination rate were statistically significant ($p < 0.05$). In contrast, LAE-9 and S-20 did not significantly affect germination compared with control group. Considering LAE-9 had a moderate positive effect on root and stem length, thus, LAE-9 is recommended for the safe surfactant.

Film composition is an important component in seed coating formulations, which could control the release of active constituent and protect seed from the stressing condition injuries [15]. The film-forming property of different film composition and their effect on seedling quality were investigated (Table 2). For film-forming property, polyacrylamide + carboxymethyl cellulose (CMC) clearly demonstrated better results in all areas in the main performance indexes than that of other three film former. Compared with the control group, there was no obvious change in seedling quality in the film former group. Among them, polyvinyl alcohol and polyacrylamide + CMC had a relatively higher positive promotion effect on the emergence rate, fresh weight, and root and stem length. Thus, the optimal effect on the seedling quality is by using polyacrylamide + CMC.

After primary screening, the optimal formula was as shown in Table 3. Based on the above optimal formula, several seed-coating agents were prepared, and the main performance indexes meet the agent requirement.

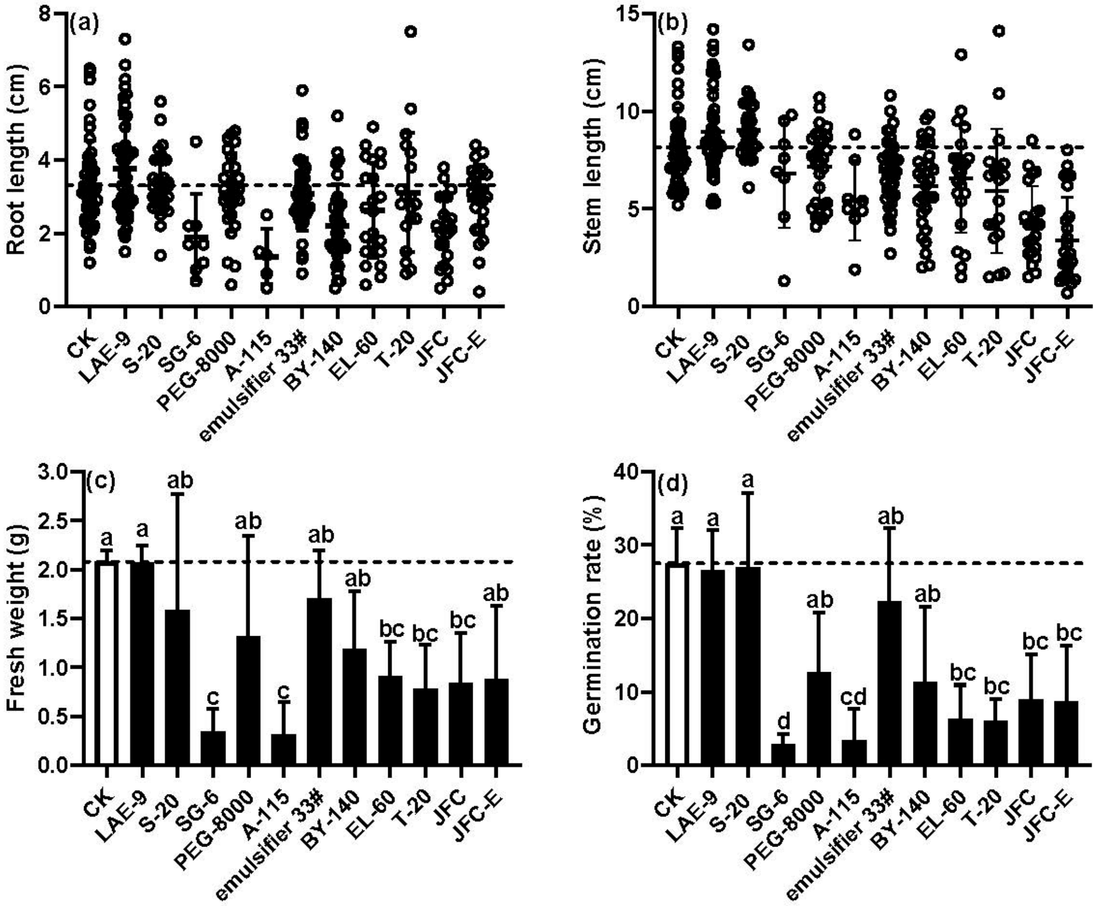

Figure 1. The effect of nonionic surfactants on the root length (**a**), steam length (**b**), fresh weight (**c**), and germination rate (**d**). Bars with different lowercase letters are significantly different (one-way ANOVA followed by Tukey's multiple comparison test; $p < 0.05$).

Table 2. The effect of different film former on seedling quality.

Composition	Dosage (%)	Seedling Quality			
		Emergence Rate (%)	Root Length (cm)	Stem Length (cm)	Fresh Weight (g)
Carboxymethyl cellulose (CMC)	3	75.3	6.17	8.11	2.67
Polyvinyl alcohol (PVA)	3	77.5	6.35	9.32	2.84
Polyacrylamide	3	72.3	6.26	8.62	2.81
Polyacrylamide + CMC	3	77.1	6.42	9.11	2.83
CK		74.3	6.23	8.64	2.70

Table 3. The main components of seed coating.

Compound	% (g/g)	Properties
Fungicides	6%	Active ingredient
LAE-9	2.65%	Nonionic surfactant
NNO	4%	Wetting dispersant
Polyacrylamide + CMC	3%	Film former
Ethylene glycol	4%	Antifreeze
Gelatin	0.225%	Thickener
Pigment red	6%	Dye
Water	74.125%	

3.3. The Comparison Result of Field Trial

The effect of seed coating agents on germination and growth of wheat in field trial was investigated (Table 4). It showed that the seed coating agent had a moderate positive effect on wheat biomass but the stem was negatively impacted and there was no effect on root length. Exceptions included 6% phenamacril · tebuconazole flowable concentrate for seed coating (FSC), which had obvious negative effect on the dry weight. The differences in wheat biomass were also observed between the different field location. Briefly, the fresh and dry weight with different seed coating agent treatment in Longkang ranged from 0.355 to 0.546 g and 0.027 to 0.046 g, respectively, while the corresponding values in Yingshang were 0.540–0.698 g and 0.065–0.099 g, respectively. This was possibly due to the effect of farming methods, wheat–maize rotations are dominant cropping systems in Yingshang whereas the farming methods of Longkang is wheat after rice. Previous studies also showed that there are differences among different farming methods in root system biomass and root system volume [20,21]. 6% pyraclostrobin · difenoconazole FSC and 6% difenoconazole ·thiram FSC were best for promoting the germination and growth of wheat in Longkang and Yingshang, respectively, which provided better wheat biomass than did the positive control, e.g., Celest Top, FSC (difenoconazole + fludioxonil + thiamethoxam, 2.2 + 2.2 + 22.6%, respectively).

The control efficiency of the seed coating agents on soil-borne diseases in field trial was also followed. In this study, we only investigated wheat sharp eyespot due to the disease occurring only one year in the field. Results showed that 6% pyraclostrobin ·thifluzamide FSC and 6% phenamacril · tebuconazole FSC effectively controlled and reduced disease severity in the Yingshang field, and the control efficiency reached 94.30% and 96.67%, respectively. In Longkang field, the control efficiency of 6% azoxystrobin · fludioxonil FSC was the highest (98.52%), followed by 6% difenoconazole · fludioxonil FSC (81.57%), whereas thiram · difenoconazole FSC was comparatively lower (43.70%).

Considering the growth parameters and control efficiency, we suggested that the use of the 6% difenoconazole · fludioxonil FSC as the seed coating agents appears to give effective control of wheat sharp eyespot meanwhile had positive promotion effect on the germination and growth of wheat in field.

Table 4. The effect of seed coating agents on germination and growth of wheat in field trial.

Experimental Sites	Agents	Main Performance Indexes of Wheat					Control Efficiency of Sharp Eyespot	
		Root Length (cm)	Stem Length (cm)	Stem Width (cm)	Fresh Weight (g)	Dry Weight (g)	Disease Index	Control Effect (%)
Longkang	6% pyraclostrobin · fludioxonil FSC	11.681 a	11.404 b	0.237 a	0.544 ab	0.042 bc	19.60 d	58.48 cd
	6% pyraclostrobin · thiram FSC	10.152 a	14.536 b	0.251 a	0.529 ab	0.043 bc	12.85 de	72.78 bc
	6% pyraclostrobin · thifluzamide FSC	11.900 a	13.124 b	0.227 a	0.545 ab	0.039 bc	20.24 cd	57.12 cd
	6% pyraclostrobin · difenoconazole FSC	10.022 a	15.211 ab	0.259 a	0.545 ab	0.046 bc	18.29 d	61.26 bc
	6% difenoconazole · fludioxonil FSC	9.019 a	12.207 b	0.198 ab	0.359 b	0.034 bc	8.70 e	81.57 ab
	6% difenoconazole · thiram FSC	10.551 a	9.377 b	0.232 a	0.382 b	0.032 bc	26.57 cd	43.70 d
	6% azoxystrobin · fludioxonil FSC	10.754 a	9.770 b	0.205 a	0.378 b	0.032 bc	0.70 g	98.52 a
	6% phenamacril · fludioxonil FSC	10.669 a	9.833 b	0.204 a	0.391 b	0.029 c	22.08 cd	53.22 cd
	6% phenamacril · tebuconazole FSC	12.871 a	10.480 b	0.244 a	0.478 ab	0.035 bc	11.19 de	76.30 ab
	CK	9.977 a	11.411 b	0.189 ab	0.355 b	0.027 c	47.20 ab	—
	Celest Top	10.702 a	10.476 b	0.230 a	0.383 b	0.034 bc	15.52 d	67.11 bc
Yingshang	6% pyraclostrobin · fludioxonil FSC	8.04 a	21.116 a	0.140 b	0.615 a	0.076 ab	30.16 bc	54.87 cd
	6% pyraclostrobin · thiram FSC	9.066 a	20.276 a	0.140 b	0.616 a	0.078 ab	37.88 bc	43.31 d
	6% pyraclostrobin · thifluzamide FSC	7.862 a	19.142 a	0.162 b	0.634 a	0.073 ab	3.81 f	94.30 a
	6% pyraclostrobin · difenoconazole FSC	9.486 a	22.186 a	0.144 b	0.641 a	0.075 ab	31.19 bc	53.32 cd
	6% difenoconazole · fludioxonil FSC	9.648 a	20.390 a	0.178 ab	0.622 a	0.099 a	12.14 de	81.83 ab
	6% difenoconazole · thiram FSC	10.57 a	21.176 a	0.190 ab	0.698 a	0.094 a	20.32 cd	69.60 cd
	6% azoxystrobin · fludioxonil FSC	9.446 a	19.816 a	0.150 ab	0.540 ab	0.073 ab	20.63 cd	69.12 bc
	6% phenamacril · fludioxonil FSC	9.24 a	19.280 a	0.180 ab	0.577 ab	0.074 ab	14.17 de	78.80 ab
	6% phenamacril · tebuconazole FSC	9.878 a	17.098 ab	0.192 ab	0.564 ab	0.065 ab	2.22 f	96.67 a
	CK	10.508 a	19.380 a	0.206 a	0.691 a	0.076 ab	66.82 a	—
	Celest Top	10.633 a	20.047 a	0.179 ab	0.603 a	0.079 ab	11.26 de	78.15 ab

Means within a column followed by the different letters (e.g, a, b, and c) are significantly different ($p < 0.05$).

4. Conclusions

In this study, we selected efficient, safe fungicides as active constituent according to the toxicity determination and safety test results. Combined with the screening of additives, we developed several wheat seed coatings. Then, through field safety compared test, the effect of seed coating on germination and growth of wheat and control efficiency were studied. We suggested that 6% difenoconazole · fludioxonil FSC can be used as an effective seed coating agent for the control of sharp eyespot of wheat in Anhui, China. However, more studies are needed on the toxicity and effect of the wheat take-all and root rot.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/8/413/s1, Table S1: Property of the nonionic surfactants.

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Conflicts of Interest: The authors declare no conflict of interest.

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