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Preparation of an Environmentally Friendly Rice Seed Coating Agent and Study of Its Mechanism of Action in Seedlings

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Abstract: Traditional rice seed coating agents (TRSCA) contain toxic components that pollute the environment and threaten human health. The use of safe, high-efficiency, and environmentally friendly seed coating agents is vital for environmental protection. We studied the production of a new, environmentally friendly rice seed coating agent and its mechanism at the seedling stage. We assess the difference in mechanism of action between the new seed coating agent and the representative TRSCAs on the market through laboratory and field experiments. Following the application of the new seed coating agent, bakanae disease was controlled at a rate of over 80.5% and insect pest feeding was controlled at a rate of 81%. More importantly, the LC_{50} value was 10 times higher than following TRSCA treatment. The coating agent can enhance the activity of plant protective enzymes (peroxidase [POD], catalase [CAT], and superoxide dismutase [SOD]) and the activity of rice seedling roots. The coating agent is antibacterial, disease preventative, deworming, safe, and environmentally protective, and results in the production of strong seedlings, suggesting it would be a good alternative to TRSCA. Our analysis found that the control effect of the seed coating on rice seedling disease was likely achieved by activating the plant protection enzymes (e.g., POD, CAT, and SOD). The effect of the coating agent on rice is likely achieved through increased root activity and the improvement of the rhizosphere micro-ecological system.

Keywords: environmentally friendly seed coating agent; natural polymer polysaccharide; rice seedling stage; mechanism

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1. Introduction

Rice is a staple food crop with the highest cultivated area in China. Increasing rice productivity is critical to ensuring food security [1]. Traditional coating technology is one of the most important ways to increase production. However, the traditional chemical seed coatings for treating rice seeds are mostly pesticide-based seed coating agents, which contain toxic insecticidal and bactericidal ingredients [2,3]. Application of the pesticide component in the coating agent not only causes phytotoxicity that impairs seedling growth and development, but it also leaves persistent pollution in farmland, which poses great harm to humans, animals, and the surrounding environment [4]. As green and organic foods are becoming increasingly important to consumers, the production of pollution-free agriculture is developing rapidly. One of the major aims in agriculture and environmental protection is to develop a safe, pollution-free, highly efficient, and environmentally friendly rice seed coating agent (ERSCA) [5].

In this study, a green polymer natural polysaccharide is used as the main active ingredient in a seed coating agent to replace the highly toxic pesticide components in TRSCA, and a new high-efficiency ERSCA is prepared. Mechanisms of action were compared between the ERSCA and the representative TRSCA on the market in both indoor and field seedling quality tests. Through an inhibition test and measurement of chlorophyll content, plant protection enzyme levels (e.g., peroxidase [POD], catalase [CAT], and superoxide dismutase [SOD]), and root activity during the rice seedling stage, the mechanism of disease

and pest prevention and improvement of seedling quality following ERSCA treatment were determined [6–8]. We performed a pest-repellent test to assess potential antifeeding effects and to analyze the mechanism of antifeeding (i.e., repelling vs. killing) [9]. The safety and environmental impact of the ERSCA were assessed using LC_{50} [10]. Therefore, this article is of great significance both in terms of theoretical research and practical applications.

2. Materials and Methods

2.1. Preparation of the ERSCA

The extraction of natural polymer polysaccharides (NPP) was performed as described by Wengang Xu [11]. The NPP was extracted from waste shrimp shells, with the main component being chitosan. Compounding was performed [12], with the ratio of natural polymer polysaccharide to deionized water to cosolvent of 1:10:0.8. The resulting mixture was stirred at 25 °C for 4.5 h at standard atmospheric pressure. Additional heating and stirring were performed (at 45 °C for an additional 5 h) to completely dissolve the NPP into a homogeneous aqueous solution. Next, the other components of the coating agent (e.g., film-forming assistant, plant growth regulator, anti-settling agent, dispersing agent, and color paste) were added to the solution at 45 °C and standard atmospheric pressure [13]. The solution was then stirred at 45 °C and standard atmospheric pressure for 5 h until all components were completely dissolved, resulting in the ERSCA finished product. The ERSCA preparation process is shown in Figure 1.

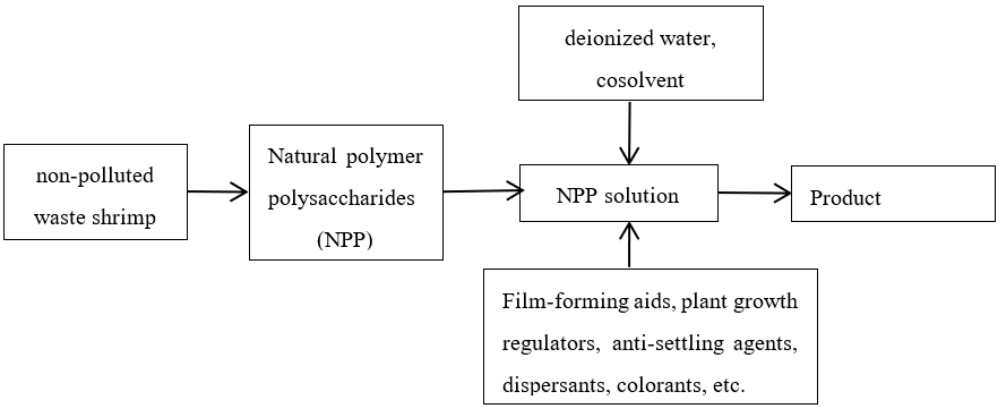

Figure 1. Process flow chart for preparing environment-friendly rice seed coating agent.

2.2. Analysis of Coating Agent Application Effects and Mechanism of Action

2.2.1. Analysis of Coating Agent Effect on the Development of Rice Seedling Disease

To study the difference between ERSCA and TRSCA treatments in controlling rice seedling disease (known as rice seedling blight), we performed an indoor year-round experiment. We assessed the ERSCA (group 1) and TRSCA (group 2) treatments separately. The traditional seed coating agent was obtained from Syngenta (China) Investment Co. The main ingredients are 37.5% methicillin, 25% fludioxonil, and 37.5% other auxiliaries. The rice seedling pathogen was applied using a spray inoculation method on all leaves, along with a blank control group [14]. A graded survey was performed according to the incidence of disease symptoms on the leaves, and the disease index was calculated [15,16].

The disease index was calculated based on the following parameters: (1) disease prevention mechanism following ERSCA application and (2) seed coating bacteriostatic activity.

Fusarium moniliforme (FM), which causes rice seedling disease, is one of the most common pathogens affecting rice seedlings. FM infection can occur from the seedling stage to the heading stage [17]. FM was the main focus of our research, and the inhibitory effect of this seed coating and traditional seed coating on FM was investigated. Colonies of rice seedlings were grown on potato dextrose agar (PDA) plates for 7 days. A bacterial culture with a diameter of 7.00 mm was excised with a puncher and placed in the middle of a new PDA plate. Endophytic bacterial strains (2-point symmetry) were placed 20 mm from both

sides of the pathogen of the bacterium, and the pathogens were used as controls. Each treatment was repeated three times and placed in a 25 °C incubator. On the 6th day of growth, the colony widths were measured, and the inhibition rate was calculated. The formula for calculating the inhibition rate is given below [18].

The change in the activity of rice protective enzymes at 0, 6, 12, 24, 48, and 72 h after pathogen inoculation was measured [19]. The activity of SOD was determined using the nitroblue tetrazolium photoreduction method; POD activity was determined using the guaiacol method; and CAT activity was determined using spectrophotometry [20–22].

2.2.2. Repellent Test of ERSCA on Insects

In this experiment, the response of brown planthoppers to seedlings treated with different seed coatings was measured using a Y-type olfactory instrument. The survey index of brown planthoppers in the field-grown seedlings after treatment with different seed coating agents was calculated. The two arms of the Y-type olfactometer and the straight tube are 20.0 cm long, the inner diameter is 2.0 cm, and the angle between the two arms is 75°. The arms of the Y-shaped tube pass through a medical rubber tube and two glass cylinders as a source of volatile matter. Seedlings treated with TRSCA were placed in a glass jar, and seedlings treated with ERSCA were placed in another glass jar [23]. Before the airflow enters the source bottle, an activated carbon filter and a distilled water bottle purify the air and increase humidity. The flow of air per arm of the Y-type olfactometer is controlled by a gas flow meter. At the time of the bioassay, the glass jar containing the plants was completely sealed with Vaseline, and the entire bioassay process was carried out at room temperature of 20 °C. An incandescent lamp was placed in front of the olfactometer and covered with a yellow cover. Insects crawl forward to the fork of the Y-shaped tube for selection toward the light source. During the bioassay, brown planthoppers are transferred into the straight tube of the Y-type olfactometer with a brush, and the insects are exposed to the two-armed odor source. When an insect moves above one of the arms and continues, it is noted as a choice for the source of the odor in that arm. However, if the insect does not make a choice, it is noted that there is no reaction, and the behavior observation is concluded.

In order to eliminate potential sources of error caused by various factors, such as the uniformity of the light intensity between the two arms of the olfactometer, the symmetry of the two arms of the olfactometer, and the interference between the brown planthoppers, we scrubbed the olfactometer with alcohol and switched the direction of its arms after every five brown planthoppers throughout the biometric process. At the same time, each of the 10 brown planthoppers was tested, and the positions of the two odor sources were exchanged. Taking 100 observations as one repeat, a total of three replicates were measured, and the results were statistically analyzed to observe the avoidance effect of the ERSCA on brown planthopper behavior [24]. The formula for calculating the avoidance rate is as follows:

$$\text{Repellent rate } (\%) = \frac{V_i - V_o}{V_t} \times 100\% \quad (1)$$

where V_i and V_o are the number of control brown planthoppers and the number of brown planthoppers in the treatment group. V_t is the total number of tested brown planthoppers in the experiment.

The formula for disease index and quasi bacteria is as follows:

$$\text{Disease index } (\%) = \frac{\sum N_d * V_i}{N_t * V_h} \times 100\% \quad (2)$$

where N_d and V_i are the number of diseased leaves and representative values. Where N_t and V_h are the number of blades and highest representative values.

$$\text{Quasi bacteria} = \frac{W_0 - W_1}{W_2 - W_3} \times 100\% \quad (3)$$

where W_0 and W_1 are blank control colony width and handing colony width. Where W_2 and W_3 are blank control colony width and control colony width at the time of inoculation, respectively.

2.2.3. Quality Assessment of Rice Seedlings following ERSCA Treatment

The seedling quality of each treatment group was examined, specifically the emergence rate, plant height, leaf age, root length, root count per 100 seedlings, hundred-grain weight, one leaf length, two leaf length, three leaf length, stem base width, and rooting force. This method is referred to as "Rice field test methods and measurement techniques [2]".

2.2.4. Assessment of the Toxicity and Safety of the Coating Agent

(1) Chemicals and materials

The tested chemical agents included the TRSCA and the ERSCA. The TRSCA was obtained from Syngenta (China) Investment Co. (Shanghai, China) and is composed of 37.5% methicillin, 25% fludioxonil, and 37.5% other auxiliaries. We tested both agents in zebrafish.

(2) Experimental method

Zebrafish were maintained at a controlled temperature of (22.5 ± 2.5) °C. The tank water was filtered using an activated carbon filter device and used after full aeration. The pH was 7.00, the dissolved oxygen was >5.8 mg/L, and the test adopted a "semi-static method". According to the results of a pre-test, the reagents were mixed with water to a total of five test mass concentrations of 1:100, 1:300, 1:500, 1:700, and 1:1000, and a blank control group was included. The test and control groups were performed simultaneously, and the liquid was changed every 24 h. The zebrafish were placed in the test and control cylinders, with 10 fish per cylinder. The test and control groups were arranged in three parallels, and each cylinder was one parallel. During the test, dead fish were removed. After treatment administration, zebrafish poisoning and deaths were observed. A glass rod was used to touch each fishtail, and if the fish did not respond, it was considered dead. Zebrafish poisoning and death were recorded at different doses and at different time points. We then calculated the LC_{50} value and 95% confidence interval for the acute toxicity of TRSCA and ERSCA in zebrafish [25,26].

(3) Toxicity classification standard

According to China's "Experimental Criteria for Environmental Safety Assessment of Chemical Pesticides", the classification criteria for toxicity levels on fish were divided. The toxicity level of four pesticides on zebrafish is shown in Table 1 [27].

Table 1. Toxicity grading standards.

Toxicity Grad	LC_{50} (96 h)/(a.i.mg L^{-1})
Very toxicity	$LC_{50} \leq 0.100$
Hightly toxicity	$0.100 \leq LC_{50} \leq 1.00$
Medium toxicity	$1.00 < LC_{50} \leq 0.100$
Low toxicity	$LC_{50} > 0.100$

3. Results

3.1. Comparison of the Effects of Different Rice Seed Coating Agents for Controlling Rice Seedling Disease

3.1.1. The Effect of the Coating Agent on Controlling Rice Seedling Disease

Seven days after administration in both treatment groups, the average control effect of the three investigations ranged from 71.23% to 90.53%. For both TRSCA and ERSCA (soaking, seed dressing with soaking, and seed dressing three treatment methods), the average control effect was higher than 50%. The results are shown in Table 2.

The control effects of the three application methods (soaking, soaking with seed dressing, and seed dressing) of the two test dressing agents were similar to those of the control agents, as shown in Table 2. The resistance of rice to the disease sexuality began to increase after the concentration increased and reached the maximum value 7 days after the best application method (soaking and seed dressing), and then gradually decreased after 14 days and 21 days.

The anti-disease effect of the ERSCA treatment methods of soaking seeds and seed dressing treatment was the slowest, and the rate of disease resistance decreased by 1.9% by the 21st day, followed by TRSCA treatment (5.04%) under soaking conditions, and the rate of decline in disease resistance was slightly lower than that of the ERSCA treatment under soaking and seed dressing conditions (1.9%), where the effect period was equivalent to that of the control agent. The disease-resistance effect was the fastest in the traditional soaking group. On the 14th day, the disease resistance effect was 65.21% and the decline rate was 6.82%; the shortest period of validity was 7–14 days and the remaining treatment period was 14–21 days. The results are shown in Table 2.

3.1.2. Experimental Results of the Repellent Effect of the ERSCA on Pests and Diseases

Seven days after administration in the three treatment groups, the average avoidance effect of the three investigations ranged from 26.31% to 56.68%. The TRSCA treatment (soaked seeds and seed dressing) and the average avoidance effect of ERSCA (soaking seeds and seed dressing) was higher than 50%, whereas the average avoidance effect of the blank group was only 26.31% (Table 3).

The avoidance effects observed in the three application methods (soaking, soaking with seed dressing, and seed dressing) of the two test dressing agents were similar to those of the control agents, which are shown in Table 2. This indicated a deterrent effect against brown planthoppers. The effect began after the concentration increased, reached a maximum at 7 days after treatment with the optimal concentration of 1:200, and then gradually decreased at 14 d, 21 d, and 28 d.

The effect of ERSCA + seed dressing treatment was the slowest, and the rate of decline in the effect of avoidance on the 28th day was 37.08%, followed by TRSCA (37.76%) under the conditions of soaking and seed dressing, and the effect of avoidance was decreased. The rate was slightly lower than following ERSCA treatment (41.08%), and the duration of the effect was comparable to that of the control agent, more than 28 days. The most effective decline in disease resistance was in the blank group. On the 14th day, the effect of avoidance was 33.36%, the rate of decline was 31.06%, the duration of the effect was the shortest (7 to 14 days), and the remaining treatment period was 14 to 21 days (Table 3).

3.1.3. Effect of ERSCA Treatment on Seedling Growth

Table 4 shows the effects of the TRSCA and ERSCA treatments on the rate of rice seedling formation and the seedling quality data after coating. It was found that ERSCA treatment significantly increased the rate of seedling formation. Compared with the control group, ERSCA soaking, seed dressing with soaking, and seed dressing increased seedling formation by 4.07%, 4.78%, and 5.66%, respectively, and the difference was significant. As shown in Table 4, after the ERSCA and TRSCA treatments were applied, the difference between the seedling formation rates was less than the difference between the germination rate and the seeding rate of the blank control rice, which indicated that the ERSCA mainly promoted seed germination. In addition, the effect of the TRSCA treatment on the seedling rate was not obvious.

Table 2. Induction and disease resistance of different seed coating agents against mites.

Treatment	Application Method	Initial Disease Index	7 Days after the Soaking Seeds		14 Days after the Soaking Seeds		21 Days after the Soaking Seeds	
			Disease Index	Induction Effect/%	Disease Index	Induction Effect/%	Disease Index	Induction Effect/%
ERSCA	Soaking seeds	9.9 ± 1.68	13.76 ± 2.75	87.32	15.36 ± 2.64	86.56	15.6 ± 2.35	81.52 f
	seed dressing	14.17 ± 1.94	13.08 ± 2.17	88.56	13.87 ± 2.71	87.13	13.12 ± 2.74	85.23 bc
	Soaking seeds + seed dressing	9.87 ± 1.57	11.66 ± 1.12	90.53	13.87 ± 1.82	88.52	14.46 ± 2.33	87.33 f
TRSCA	Soaking seeds	9.85 ± 2.01	15.43 ± 3.66	72.03	12.59 ± 3.08	65.21	10.84 ± 4.5	61.11 e
	seed dressing	12.51 ± 1.68	14.19 ± 1.52	73.5	13.62 ± 1.65	72.56	10.58 ± 1.97	71.66 b
	Soaking seeds + seed dressing	11.71 ± 1.53	16.83 ± 1.44	74.6	11.31 ± 1.9	73.78	10.66 ± 2.92	71.55 bc
CK	No treatment seed	14.71 ± 3.86	28.35 ± 4.14	—	32.94 ± 4.23	—	45.43 ± 4.75	—

Note: The data in the table are the average of three replicates. The lowercase letters in the same column indicate that the difference is significant at the 5% level, the same below.

Table 3. Evasive effect of different seed coating agents on brown planthoppere.

Treatment	Method	1:500 7 Days after the Soaking Seeds				1:1000 7 Days after the Soaking Seeds				1:1500 7 Days after the Soaking Seeds			
		A_1	A_2	A_0	(%)	A_1	A_2	A_0	(%)	A_1	A_2	A_0	(%)
ERSCA	S_1	33	41	26	67	31	44	25	69	32	42	26	68
	S_2	26	46	28	74	24	49	27	76	24	47	29	76
	S_3	20	52	28	80	18	55	27	82	19	53	28	81
TRSCA	S_1	36	39	25	64	34	41	25	66	35	40	25	65
	S_2	29	44	27	71	29	46	25	71	29	46	25	71
	S_3	23	50	27	77	20	52	28	80	21	51	28	79

Note: S_1: soaking seeds; S_2: seed dressing; S_3: soaking seeds + seed dressing; A_1: The number of selecting processing groups; A_2: the number of selecting blank groups; A_0: the number of not selecting; avoidance effect %.t.

Table 4. Effect of different seed coating agents on rice seedling growth rate and seedling quality.

Treatment	Seedling Rate	Leave Age	Seedling Height	Leaf Erection	Leaf Length	Root Length	Total Roots per Pant	White Roots per Pant	Cauline Basilar Width	Fresh Weight of Shoot	Dry Weight of Shoot
ERSCA	88.02 ± 2.09 a	2.92 ± 0.01 a	19.27 ± 2.57 a	3.89 ± 0.03 a	11.71 ± 0.15 a	12.23 ± 1.47 a	6.22 ± 0.18 a	3.33 ± 0.19 a	2.01 ± 0.34 a	1.20 ± 0.09 a	0.32 ± 0.03 a
ERSCA	88.73 ± 2.09 a	2.98 ± 0.01 a	19.66 ± 2.57 a	3.89 ± 0.03 a	11.97 ± 0.15 a	12.89 ± 1.47 a	6.99 ± 0.18 a	3.98 ± 0.19 a	2.06 ± 0.34 a	1.28 ± 0.09 a	0.34 ± 0.03 a
ERSCA	89.61 ± 2.09 a	3.01 ± 0.01 a	20.97 ± 2.57 a	4.01 ± 0.03 a	12.16 ± 0.15 a	13.78 ± 1.47 a	7.63 ± 0.18 a	4.96 ± 0.19 a	2.68 ± 0.34 a	1.50 ± 0.09 a	0.37 ± 0.03 a
TRSCA	87.99 ± 2.09 a	2.92 ± 0.01 a	19.03 ± 2.57 a	3.83 ± 0.03 a	11.60 ± 0.15 a	12.10 ± 1.47 a	6.01 ± 0.18 a	3.26 ± 0.19 a	1.99 ± 0.34 a	1.17 ± 0.09 a	0.31 ± 0.03 a
TRSCA	88.41 ± 2.09 a	2.98 ± 0.01 a	19.15 ± 2.57 a	3.87 ± 0.03 a	11.95 ± 0.15 a	12.35 ± 1.47 a	6.09 ± 0.18 a	3.66 ± 0.19 a	1.99 ± 0.34 a	1.18 ± 0.09 a	0.33 ± 0.03 a
TRSCA	88.71 ± 2.09 a	2.99 ± 0.01 a	20.16 ± 2.57 a	3.97 ± 0.03 a	12.03 ± 0.15 a	12.65 ± 1.47 a	6.23 ± 0.18 a	3.95 ± 0.19 a	2.28 ± 0.34 a	1.20 ± 0.09 a	0.34 ± 0.03 a
CK	83.95 ± 4.41 a	2.91 ± 0.03 a	18.61 ± 2.65 a	3.80 ± 0.03 a	11.08 ± 0.59 a	11.69 ± 1.65 a	6.09 ± 0.11 a	3.50 ± 0.10 a	1.91 ± 0.23 a	1.17 ± 0.10 a	0.30 ± 0.03 a

Note: The mean difference with the same letter in the same column in the table is not significant ($p > 0.05$).

Stem base width is an important indicator in describing the height of the seedling array. The experimental results of applying a seed coating agent showed that the ERSCA and TRSCA treatments increased the stem base width of the seedling, and the ERSCA soaking, seed dressing with soaking, and seed dressing increased the stem base width by 0.1 cm, 0.15 cm, and 0.77 cm, respectively. The difference was significant.

The accumulation of dry matter can directly reflect the growth potential and growth of the seedlings, and it also affects rice yield. The experimental results in Table 4 show that both TRSCA and ERSCA can increase the dry matter accumulation of seedlings. Compared with the control group, ERSCA soaking, seed dressing with soaking, and seed dressing increased 0.02 g, 0.04 g, and 0.07 g, respectively. The difference was significant, indicating that the ERSCA treatment was beneficial to the dry matter accumulation of the seedlings.

3.1.4. Toxicity and Safety of Different Rice Seed Coating Agents

The toxicity test results of TRSCA and ERSCA exposures at the same concentration (1:1000) on zebrafish at 24 h, 48 h, 72 h, and 96 h are shown in Tables 5 and 6. The zebrafish did not die in the 5 test concentration groups of ERSCA at 24 h, 48 h, 72 h, and 96 h, indicating that the LC_{50} of the zebrafish was greater than that of the self-made seed coating for 24 h, 48 h, 72 h, and 96 h, 100 aimg/L. The LC_{50} of zebrafish following TRSCA exposure was 7.04 mg/L and 5.14 mg/L for 24 h and 48 h. Thus, in zebrafish, TRSCA exposure is more toxic than ERSCA exposure. In view of the possible impact of dilution on the toxicity and safety of rice seed coating agents, we used concentrations of 1:700, 1:500, 1:300, 1:100 for dilution. However, the results of the determination of toxicity were the same as that of dilution at 1:1000 concentration (Tables 5 and 6). This further confirmed that TRSCA exposure is more toxic than ERSCA exposure to zebrafish.

Table 5. LC_{50} of TRSCA on zebrafish was determined by semi-static method.

Treatment	Time/h	Toxicity Regression Equations	Chi-Square	Sig	LC_{50} 95%CL/(mg/L)
TRSCA (1:1000)	24	Y = 13.704x − 10.862	0.756	0.943	7.04 (7.00~6.43)
	48	Y = 13.715x − 10.855	0.755	0.958	5.14 (5.45~5.96)
	72	Y = 14.646x − 10.634	0.655	0.943	4.24 (4.12~4.52)
	96	Y = 14.119x − 10.204	0.611	0.958	2.62 (2.07~2.88)
TRSCA (1:700)	24	Y = 9.592x − 7.603	0.725	0.895	4.928 (4.00~4.98)
	48	Y = 9.600x − 7.598	0.725	0.910	3.598 (3.45~3.96)
	72	Y = 10.252x − 7.443	0.628	0.895	2.968 (2.12~2.97)
	96	Y = 9.883x − 7.143	0.586	0.910	1.834 (1.07~1.98)
TRSCA (1:500)	24	Y = 6.852x − 5.431	0.733	0.905	3.52 (3.00~3.93)
	48	Y = 6.857x − 5.427	0.732	0.919	2.57 (2.02~2.76)
	72	Y = 7.323x − 5.317	0.635	0.905	2.12 (2.12~2.52)
	96	Y = 7.059x − 5.102	0.592	0.919	1.31 (1.07~1.48)
TRSCA (1:300)	24	Y = 4.111x − 3.258	0.740	0.886	2.112 (2.00~2.43)
	48	Y = 4.112x − 3.256	0.739	0.900	1.542 (1.45~1.96)
	72	Y = 4.393x − 3.190	0.641	0.886	1.272 (1.01~1.29)
	96	Y = 4.235x − 3.061	0.598	0.900	0.786 (0.17~0.93)
TRSCA (1:100)	24	Y = 2.741x − 2.172	0.748	0.886	0.704 (0.19~1.22)
	48	Y = 2.743x − 2.171	0.747	0.900	0.514 (0.43~1.01)
	72	Y = 2.929x − 2.126	0.648	0.886	0.424 (0.31~3.22)
	96	Y = 2.823x − 2.040	0.604	0.900	0.262 (0.17~0.33)
CK	No treatment seed	-	-	-	-

Table 6. LC_{50} of ERSCA on zebrafish was determined by semi-static method.

Treatment	Time/h	Toxicity Regression Equations	Chi-Square	Sig	LC_{50} (95%CL)/(mg/L)
(1:1000) ERSCA	24	Y = 2.711x + 1.010	0.388	0.943	6.2 (6.00~6.43)
	48	Y = 3.745x + 2.178	0.309	0.958	5.68 (5.45~5.96)
	72	Y = 4.119x + 1.111	0.388	0.943	5.32 (5.12~5.52)
	96	Y = 5.130x + 2.419	0.309	0.958	5.28 (5.07~5.48)
(1:700) ERSCA	24	Y = 1.897x + 0.707	0.388	0.895	6.2 (6.00~6.43)
	48	Y = 2.621x + 1.524	0.309	0.910	5.68 (5.45~5.96)
	72	Y = 2.883x + 0.777	0.388	0.895	5.32 (5.12~5.52)
	96	Y = 3.591x + 1.693	0.309	0.910	5.28 (5.07~5.48)
(1:500) ERSCA	24	Y = 1.355x + 0.505	0.388	0.905	6.2 (6.00~6.43)
	48	Y = 1.872x + 1.089	0.309	0.919	5.68 (5.45~5.96)
	72	Y = 2.059x + 0.555	0.388	0.905	5.32 (5.12~5.52)
	96	Y = 2.565x + 1.209	0.309	0.919	5.28 (5.07~5.48)
(1:300) ERSCA	24	Y = 0.813x + 0.303	0.388	0.886	6.2 (6.00~6.43)
	48	Y = 1.123x + 0.653	0.309	0.900	5.68 (5.45~5.96)
	72	Y = 1.235x + 0.333	0.388	0.886	5.32 (5.12~5.52)
	96	Y = 1.539x + 0.725	0.309	0.900	5.28 (5.07~5.48)
(1:100) ERSCA	24	Y = 0.542x + 0.202	0.388	0.886	6.2 (6.00~6.43)
	48	Y = 0.749x + 0.4356	0.309	0.900	5.68 (5.45~5.96)
	72	Y = 0.823x + 0.222	0.388	0.886	5.32 (5.12~5.52)
	96	Y = 1.026x + 0.483	0.309	0.900	5.28 (5.07~5.48)
CK	No treatment seed	-	-	-	-

3.1.5. Analysis of the Mechanism of ERSCA Disease Prevention

(1) Comparative experiment on the antibacterial effect of different rice seed coating agents

The comparison test results of the antibacterial effects of different seed coating agents are shown in Table 7.

Table 7. Results of the bacteriostatic effects of different seed coating agents.

Seed Coating Type	Antibacterial Rate against Pathogens (%)
Environmentally friendly seed coating	87.82 ± 1.3 a
Traditional seed coating	84.36 ± 0.9 b

Note: The data in the table is the mean ± standard deviation. Different lowercase letters in the same column indicate that there is a significant difference between the different treatments at the 0.05 level.

As can be seen from Table 7, the common pathogens that harm rice growth were inhibited by the ERSCA treatment, and the inhibition rate reached 87.82%. The seed coating agent has an inhibitory effect on rice diseases, and the bacterial growth rate is higher than following TRSCA treatment.

(2) Effect of different seed coating agents on SOD enzyme activity in rice leaves

The dynamic changes in SOD activity following the inoculation with pathogens within 72 h after treatment with different seed coatings are shown in Figures 2 and 3.

As can be seen from Figure 3, the SOD enzyme activity increased rapidly after inoculation of rice seedlings with FM and reached the highest peak after 12 h following treatment, and then SOD activity began to decrease, and its activity was further decreased after 48 h.

After treatment with ERSCA + FM, the SOD activity in rice increased rapidly after 0–6 h, reached its highest peak at 6 h, then began to decrease, and its activity began to increase again after 24 h. After FM inoculation of rice treated with TRSCA, the activity of SOD enzymes did not change significantly compared with the control group (CK).

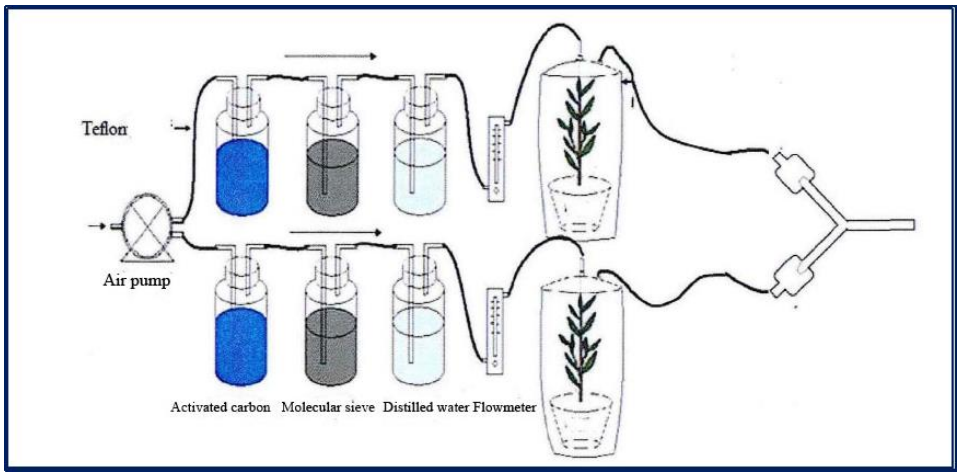

Figure 2. Schematic diagram of the Y-type olfactometer.

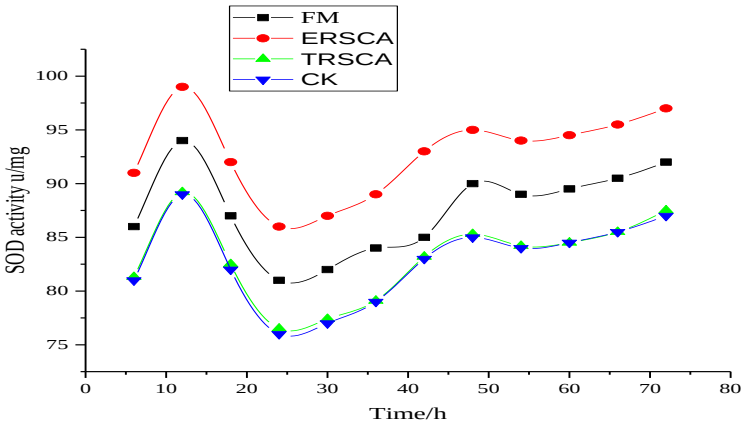

Figure 3. Comparison of effects of different seed coatings on the SOD activity of rice leaves.

As the first line of defense for plant protection enzyme systems, SOD mainly exists in three forms: Cu•Zn-SOD, Mn-SOD, and Fe-SOD. A large number of free amino groups are distributed in the NPP molecular chain. It can chelate with trace elements such as zinc, iron, copper, and manganese in the soil, which is more conducive to the absorption of these trace elements by crops and promotes the synthesis of SOD, thus increasing the SOD content and activity. The SOD activity transformation trend is shown in Figure 3. Therefore, the use of environmentally friendly seed coating agents is more conducive to the improvement of SOD activity in rice and rice disease prevention.

(3) Effect of different coating agents on the change of POD enzyme activity in rice leaves

The dynamic changes of POD enzyme activity following FM inoculation within 72 h after coating with different seed coatings are shown in Figure 4.

It can be seen in Figure 4 that the POD enzyme activity of rice seedlings increased after inoculation of rice leaves and reached its highest peak 24 h after treatment, then the specific activity of the POD enzyme began to decrease, and its activity was further decreased after 48 h. After treatment with ERSCA and FM inoculum, POD enzyme activity increased rapidly and reached its highest peak at 24 h. Then the specific activity of the POD enzyme began to decrease, and its activity began to increase after 48 h. After treatment of rice with TRSCA, POD enzyme activity did not change significantly compared with the control CK.

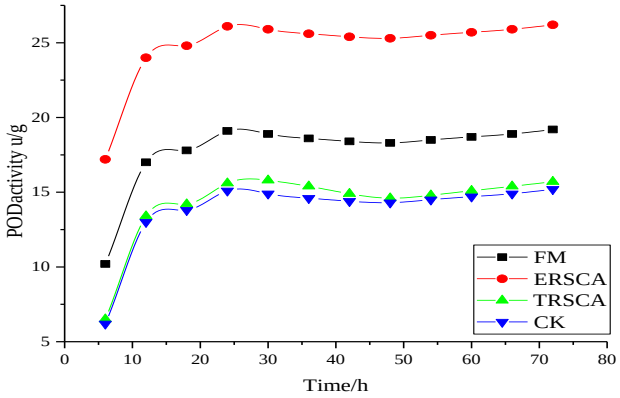

Figure 4. Effects of different seed coatings on POD enzyme activity in rice leaves.

(4) Effect of different coating agents on the changes of CAT enzyme activity in rice leaves

The dynamic changes in CAT enzyme activity following FM inoculation within 72 h after coating with different seed coatings are shown in Figure 5.

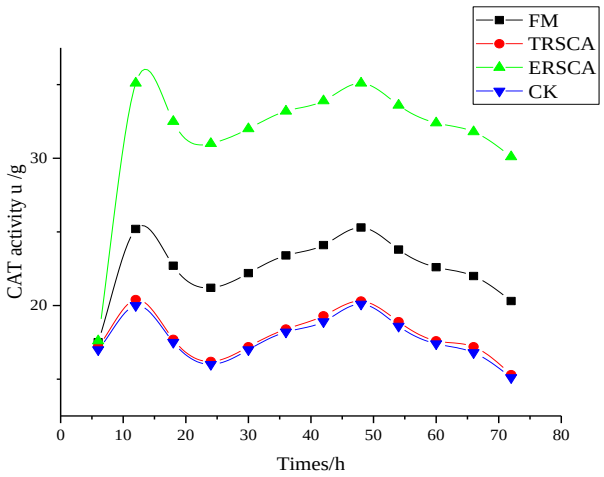

Figure 5. Effects of different SCAs on the changes in CAT enzyme activity in rice leaves.

It can be seen in Figure 5 that the CAT activity in rice seedlings increased after FM and reached the highest peak after 12 h following the treatment, and then CAT activity began to decline gradually. After treatment with ERSCA and FM, CAT enzyme activity increased rapidly. After 12 h, the activity of CAT began to decrease. After 24 h, its activity began to increase, reached a peak, and then began to fall after 48 h. Compared with CK, the POD activity of rice treated with TRSCA did not change significantly.

After inoculation with FM, the content of SOD, POD, and CAT in the rice leaves of different treatment groups was different at the same time (Figures 3–5). When plants are attacked by diseases, they often resist the invasion of the disease by increasing the content of SOD, POD, and CAT and strengthening the coordination between the three plant protection enzymes, which would explain our observation that the three enzymes in the treatment group increased after FM inoculation. Compared with CK, the content of plant protective enzymes in rice leaves in the TRSCA group did not change much, suggesting that the TRSCA treatment did not activate the plant protection enzymes but may have instead killed the pathogen on contact to control the disease. However, the content of plant protective enzymes in rice leaves in the ERSCA treatment group was significantly higher than that of the FM group. We can conclude that ERSCA treatment can induce resistance by activating enzymes that prevent and control the disease. Induced resistance is one of the functions of the plant immune system and has the benefits of being non-specific, systemic, persistent, and pollution-free.

Therefore, ERSCA treatment can induce disease resistance in rice seedlings and increase the content and activity of SOD, POD, and CAT to prevent disease. In addition, the use of this environmentally friendly seed coating is a new and important way to prevent and control modern plant diseases.

4. Conclusions and Discussion

(1) Compared with TRCSA, the new high-efficiency and environmentally friendly rice seed coating agent with NPP as its main active ingredient has many advantages, such as disease prevention, insect control, promotion of strong seedlings, safety, and environmental protection [28].

(2) The coating agent is safer and more environmentally friendly than TRCSA. TRCSA may suppress microbial growth by killing bacteria, whereas ERCSA treatment reduces rice seedling disease by means that are likely to be bacteriostatic rather than bactericidal. The application of ERCSA can induce resistance in rice plants, prevent disease by improving the activity of protective enzymes in seedlings, and has the benefits of being non-specialized, systemic, durable, and pollution-free. In addition, the coating agent protects pests by means of repelling rather than killing them, which can both protect biodiversity and protect rice from pests [29].

(3) The coating agent improves the quality of rice seedlings by increasing seed emergence rate, root activity, and enzyme activity in leaves. The seed coating can promote the synthesis of SOD, POD, and CAT enzymes in rice leaves and activate enzymes to induce disease resistance in rice, which can prevent disease while controlling and improving the quality of rice seedlings. The application of ERSCA could enhance photosynthesis by increasing the chlorophyll content, thus increasing rice yield [30]. In addition, because of the increase in POD activity, the damage caused by oxygen free radicals may be reduced, and leaf senescence can be slowed, thus promoting crop growth and increasing yield [31].

(4) Compared with TRCSA, ERCSA is a veritable green pesticide. The toxicity of ERSCA is much lower than that of TRSCA, and the EC_{50} is more than 10 times that of the conventional seed coating agent (EC_{50} > 5000 mg/kg) [32].

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