

Study on the Preparation and Increasing Production Mechanism of a Novel Environmentally Friendly Cotton Seed Coating Agent

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Abstract

A novel environmentally friendly cotton seed coating agent was prepared from natural polysaccharide, fertilizer and microelement etc, which coating of cotton achieved good effect on increasing and the obvious antifeedant effect. The results showed that the yield of cotton treated with environmentally friendly cotton seed coating agent was enhanced by 9.47%, its cost was decreased by 35% and it was much more safe and environmentally friendly, when compared to the traditional cotton seed coating agent. So it has three characteristics of high yield, less cost and friendly environment.

Keywords: Environmentally friendly material; Cotton seed coating agent; Antifeedant; Germination energy (GE); Germination rate (GR)

Introduction

Cotton seeds treated with seed coating agent can effectively prevent cotton from seedling state diseases, promote seedling growth and improve cotton yield [1]. Cotton seed coating agent is the main approach to prevent cotton from seedling rhizoctonia solani [2]. But most of the currently used cotton seed coating agents have serious poison and pollution to the human body and the environment [3], the valid components preventing cotton from rhizoctonia solani in cotton seed agents are deleterious and harmful contamination, such as phorate, carbofuran, carbendazim etc [4]. Therefore, how to develop a novel environmentally friendly cotton seed coating agent has become an important project being waiting for solution urgently in the current agriculture and the environmental protection field [5]. After a lot of researches, we finally develop a novel environmental protection type cotton seed coating agent composed by taking natural macromolecular material as main material, accompanying with film-forming agent, dispersant, trace fertilizer and microelement. Compared with the traditional cotton seed coating agents, the results showed that the yield of cotton treated with environmental friendly cotton seed coating agents has enhanced by 13.1%, the cost of seed coating agent is decreased by 29%, when compared to the traditional cotton seed coating agent. So it has the obvious antifeedant effect and it is safe, innocuous and non-contaminative, having obvious economic and environmental benefits.

Materials and Methods

Main experimental apparatus

Constant temperature and humidity incubator (WS-01, Hubei huangshi hengfeng medical apparatus limited company), electronic scales (FA2004, Shanghai yuefeng apparatus limited company), high-pressure steam sterilization pot (YXQ-SG46-48 SA, Shanghai bosun shiye limited company), temperature psychrometer (STH950, SUMMIT Corporation), electronic constant speed blender (GS28B, Shanghai an-ting electronic apparatus factory in), germination box (30cm×12cm, Hubei Agriculture Office).

Main experimental reagents

Natural polysaccharide, sodium hydroxide, borax, glycol, potassium dihydrogen phosphate, bluestone, zinc sulfate etc (all these experimental reagents are analytically pure, Wuhan changjiang chemical plant), pigment (Guangdong Shantou Mingde food additives limited company), cotton seeds (Hubei provincial seed group company), traditional seed coating agent Celest (Switzerland Xianzhengda Crop Protection Limited company).

Experiment Methods

Designing principle of formulas of cotton seed coating agent

The formulas of the cotton seed coating agent are designed according to the three greatest principles which are low price, environmental protection and high yield. The formulas of cotton seed coating agent are designed under the precondition that the material cost of cotton seed coating agent is lower than that of traditional seed coating agent selling on the market (about 20yuan·kg⁻¹). And then these novel seed coating agents are compared with traditional ones in various performance parameters in the same conditions. If certain formulas are found that main performance parameters are better than traditional ones, they are picked out primarily, and then optimized, finally the best formulas of seed coating agents are confirmed whose performance price ratio and environmental friendship are better than traditional ones.

Preparation of seed coating agents

The aqueous solution of sodium hydroxide, bluestone, zinc sulfate

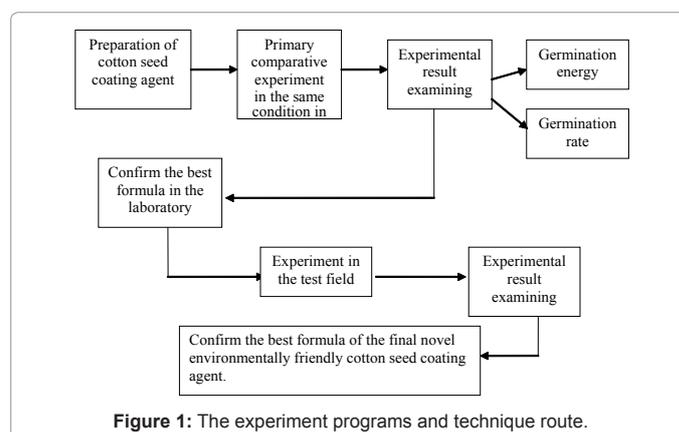


Figure 1: The experiment programs and technique route.

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and other elements were blended with natural polysaccharide and other components in certain proportion and mixed round in the electronic constant speed blender for two hours with a speed of 1000 r/min, and then we got the finished product of the novel and environmentally friendly cotton seed coating agents.

Comparative experiment of the cotton seed coating agent in the laboratory: Comparative experiment of the cotton seed coating agent in the laboratory is as follows: the same cotton seeds were treated by coating with environmentally friendly seed coating agents and conventional ones respectively, and then put in the constant temperature and humidity incubator under the same condition. Experimental result of the germination energy and germination rate should be observed and compared every day. Concrete process is as follows:

1. Preparation of coating seed

Acid-delinted seeds were treated with the environmentally friendly cotton seed coating agents and traditional ones respectively with a proportion of 1:50, airing for 20 minutes under the room temperature, and then they can be sowed. In addition, untreated seeds were used as control.

2. Germination experiment

The sand and water was mixed evenly with a certain proportion, the sand was filled in the germination box, and the thickness of the sand was a half height of the germination box. The treated seeds were sown in the sand evenly. All germination experiments were carried out using four replicates of 50 seeds each. Seeds were away from the surface of sand about 1 cm. The germination boxes were placed in the Constant temperature and humidity incubator setting the temperature for $(28\pm 1)^\circ\text{C}$, humidity for 85%RH. Germination circumstance should be investigated every 24 hours. According to national seed examination rules (ISTA 2004), the cotton growth period of germination energy is 4 day, the ultimate germination rate of seeds is 12 days, germination energy and germination rate should be observed and jotted down every day. The germination energy and germination rate were calculated from the two equations:

$$\text{Germination energy}(\%) = \frac{X}{N} \times 100$$

$$\text{Germination rate}(\%) = \frac{Y}{N} \times 100$$

Where X is the number of germinating seed in the beginning of germinating period (4days) Y is the number of germinating seed in the end of germinating period (12days), N is the total number of experimental seeds.

The germination energy and germination rate were reckoned through above two calculation formulas, among them the formula of seed coating agent which two indexes preponderating over traditional one and attaining the max is considered as the best formula of comparative experiment in the laboratory.

The antifeedant experiments

According to the biological pesticide indoor experiment standards of pesticides, using artificial feed mixing method of medicine. The methods are as follows:

Weigh the new production of artificial feed, and according to per 100 grams feed mix seed coating as 8mg, 10mg and 13.3 mg. All treatments were mixed with artificial diet been weighed in the weight ratio of 1:20. When the treated diet curdled, it will be cut into smaller

pieces. The fractions were placed in an insect plate with 24 1.5cm \times 1.5 cm holes, respectively. The plates, each containing fourth-instar larvae, were covered with lid and placed in an incubator at dark at temperature of $25\pm 1^\circ\text{C}$ and 75%-85%. The remaining diets (control and treated) were not going to be weighted until 48h later. Three replicates were maintained for each treatment. The test was valid only the mortality of larvae within 5%. To evaluate the feeding behavior a "feeding deterrence index" was calculated as follow:

$$FDI(\%) = \frac{C-T}{C} \times 100$$

Where C and T represent the amounts eaten of control and treated diets, respectively.

Field trials

Field trials was carried out to test whether the efficacy of above-mentioned several better formulas obtained in the laboratory were better than traditional one and their effect on the yield. The cotton seeds for examination were coated with the better formulas obtained in the laboratory and traditional one in the proportion of 1:50 (w/w), and then dried by airing to prepare for use. The experiment was conducted at the e'zhou experimental station, Hubei Provincial Seed Group Company. The treatments were arranged in a randomized block design with three replicates of each treatment.

Results and Discussion

Primary experiment results in the laboratory

CK was uncoated seed as control, Celest was the traditional cotton seed coating agent, A1-A8 were environmentally friendly cotton seed coating agents prepared by ourselves. The primary purpose was to select better environmentally friendly cotton seed coating agent preponderating over traditional Celest in performance price ratio. Primary experiment results in the laboratory were showed in Table 1.

It can be seen from Table 1, that the main indexes of seed coating agents A2, A5, A7 exceeded over traditional Celest. A2 was the best among them, its germination energy and germination rate was enhanced by 15.23%, 13.03% respectively when compared to Celest, but its cost decreased by 32.4%. So A2 was considered as the primary best seed coating agent in the laboratory.

Optimization experiment in the laboratory

Based on the formula of seed coating agent A2, by changing two main impact factors such as pH and the concentration of natural polysaccharide in the formula to optimize A2 and find out several

Number of Seed coating agent	GE/%	GR/%
A1	78.07e	92.13d
A2	89.4a	96.03a
A3	74.83g	87.8f
A4	79.8d	89.4e
A5	85.47b	92.7bc
A6	83.2c	93.13b
A7	85.07b	92.5cd
A8	76.13f	87.1g
CK	72.3i	80.73i
Celest	74.17h	83h

Averages in column separated by Duncan's new multiple range test, 5% level

Table 1: Primary experiment results in the laboratory.

pH	GE/%	GR/%
4	67.43g	80.7g
4.5	75.07e	83.46f
5	77.5d	90.23d
5.5	80.07b	93.36b
6	82.43a	95.73a
6.5	79.73c	91.9c
7	72.4f	85.13e

Averages in column separated by Duncan's new multiple range test, 5% level

Table 2: Effect of pH value on main parameters of seed coating agent.

concentration of natural polysaccharide	GE/%	GR/%
0.1	67.47f	77.53f
0.2	72.4e	86.53e
0.5	82.5c	87.76d
1	86.9a	97.07a
1.5	84.36b	92.33b
2	80.4d	89.93c

Averages in column separated by Duncan's new multiple range test, 5% level

Table 3: Effect of concentration of natural polysaccharide on the main parameters of seed coating agent.

Seed coating agent	GE/%	GR/%
B2-1	89.3a	97.8a
CK	70.23c	82.07c
Celest	75.4b	92.27b

Averages in column separated by Duncan's new multiple range test, 5% level

Table 4: Result of Optimization experiment and comparison with CK and Celest.

Potions name	Every 100 grams of artificial feed contains	Number of insect	Amounts of diet eaten (g)	FDI (%)
Seed Coatings	13.3mg	24	0.42d	81.42a
	10mg	24	0.79c	64.71b
	8mg	24	1.12b	50.44c
white space handling	-	24	2.26a	-

Averages in column separated by Duncan's new multiple range test, 5% level

Table 5: Antifeedant effect of seed coatings treated diet by fourth-instar *Agrotis ypsilon* Rottemberg over 48 hours.

Seed coating agent	GE/%	Per mu yield/kg	Cost of seed coating agent/\$
B2-2	98.17a	266	2.34
CK	82.33c	228	-
Celest	92.17b	243	3.6

Averages in column separated by Duncan's new multiple range test, 5% level

Table 6: The comparison results of field experiment.

better formulas of seed coating agent which performance price ratio surpassed traditional one in the laboratory.

Influence of pH value on main parameters of seed coating agent

Influence of pH value on main parameters of seed coating agent was shown in Table 2.

As can be seen in Table 2, the effect was the best when pH value was 6. In the pH value range from 4 to 6, the higher the pH value is, the higher germination energy and germination rate shows. The germination energy and germination rate reached the maximum at pH 6. When pH was above 6, with the increasing of the pH value, germination energy and germination rate declined. Therefore the best pH value of cotton seed coating agent was 6. The reason was that when the pH value was 6, the protective film made by seed coating agent at

the surface of the seeds combined with seed coat more tightly and did not change various functions of seed inner, so when the pH value was 6, the film had better permeability, it can make sure supply ample oxygen and water when seed was sprouting, providing a beneficial condition for the better growth of seed.

Influence of the concentration of natural polysaccharide on the main parameters of seed coating agent

Influence of the concentration of natural polysaccharide on the main parameters of seed coating agent was test by adjusting the concentration of natural polysaccharide and keeping other components and dosage unchanged on the base of seed coating agent A2 in the above formula (pH = 6, the concentration of natural polysaccharide = 1%). The result of the effect on the main parameters can be seen in Table 3.

It can be seen from Table 3, the concentration of natural polysaccharide had obvious influence on the germination energy and germination rate, when the concentration of natural polysaccharide ranged from 0.1% to 1%, the germination energy and germination rate increased with the increasing of the concentration, and reached the maximum when the concentration of natural polysaccharide was 1%. While the concentration of natural polysaccharide was above 1%, the two indexes began to decline. So, the best concentration of natural polysaccharide was 1%. This was because that the natural polysaccharide solution had a film forming ability. In the process of film-forming, the concentration of natural polysaccharide solution was too high, the liquidity of film isn't good, the film made was asymmetric; The concentration was too low, the bore structure of the film was loose, separating efficiency was low, having an influence on the film function.

The comparative result of optimization formula B2-1, CK and Celest

The comparative result of the optimization formula B2-1, uncoated (CK) and traditional seed coating agent (Celest) is shown in Table 4.

It can be seen from Table 4, the germination energy and germination rate of the formula B2-1 after optimization had increased by 19.07%, 25.63% respectively when compared to CK; Comparing with traditional cotton seed coating agent Celest, the two main indexes had increased by 13.9%, 5.53% respectively, while the cost had decreased by 31.2%, so it had obvious high cost-performance.

Antifeedant effect of seed coatings

Anitfeedant activity of seed coatings is presented in Table 5. As

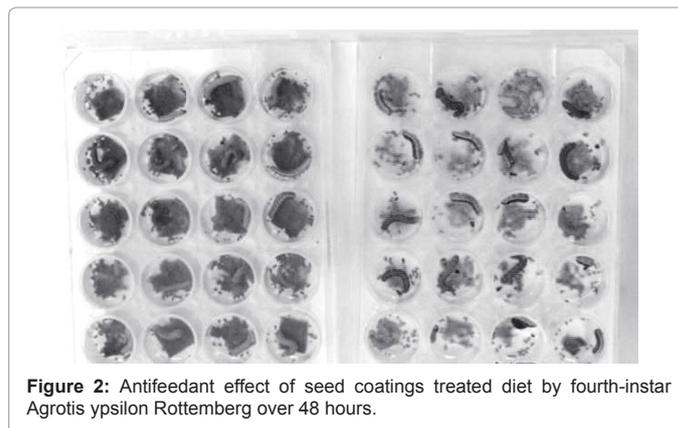


Figure 2: Antifeedant effect of seed coatings treated diet by fourth-instar *Agrotis ypsilon* Rottemberg over 48 hours.

shown in Figure 2 and Table 5, the antifeeding rate of artificial feed contains 13.3mg, 10mg, 8mg is 81.42%, 64.71%, 50.44%. It showed that antifeedant effect can be seen obviously, in which antifeeding 13.3 mg makes antifeeding effect most obviously.

The comparative result of field trials

Field trials were carried out with formulas (including B2-1) whose price ratio was better than the traditional seed coating agent Celest, adjusting the better formula further on the base of field experiment and finally getting the best formula of field test B2-2. The comparative result was shown in Table 6.

It can be seen from the Table 6, the germination rate and per mu yield of the best formula of field test B2-2 had increased by 15.84% and 16.7% respectively when compared to the uncoated cotton seed (CK), and had enhanced by 6% and 9.47% respectively comparing with the traditional seed coating agent Celest, while the cost had declined by 35%. It had indicated that the germination energy and germination rate in the laboratory were consistent with the trend of increasing yield in field experiment.

The discussion of the mechanism of antifeedant effect

Main ingredients of seed coatings is natural polymer polysaccharide, which can improve plant pest and disease resistance capability, and promote plant's growth, crop yield and quality, it's through inducing plants and immune resistance has enhance immunity, promote the growth and friendly to environment, safety, and no residue [6,7]. natural polymer polysaccharide can combining with protein receptors which on the plant cell membrane, when the receptor proteins in the membrane once receives of plant activate protein signal, stimulate resistance signal molecules such as Ca^{2+} , H_2O_2 , jasmonic acid, etc. Through the signal transduction, stimulate resistance gene expression, resistant material such as plant antitoxin, chitinase, phenylalanine solution ammonia enzyme, polyphenol oxidase, peroxidase, catalase, while activate the immune to plant disease process of salicylic acid and jasmonic acid etc, all of those can achieve a antifeedant effect.

Antifeedants effects might have direct influence on animal's taste, then transfer to taste sensors of the central nervous system; finally feeding centers stop the animal's feeding behavior. It also may directly affects the animal's nervous system, causing the nervous system, abnormal discharge from animals to get correct taste of information, in order to make them not to eat properly.

The discussion of the mechanism of increasing yield

Firstly, the natural polysaccharide used in the seed coating agent is of good film-forming ability. When cotton seeds are treated with seed coating agent, they can form a dense seed protective film on the surface of the seed. The protective film is a kind of semi permeable membrane and it can not only keep the humidity of the seed but also absorb the humidity of earth in the soil, providing adequate necessary humidity for seed pullulating, accordingly improving seed of germination and seedling [8].

Secondly, because the film of natural polysaccharide has good permeability, it can keep O_2 from getting into film inner, restrict CO_2 going outside of the film, keep the CO_2 of higher concentration inside the film, restrain the respiration of seed and make the internal nutrient consumption of seeds fell to the lowest.

Thirdly, the beneficial microelements of the plant growth are

appended in the seed coating agent, those beneficial microelements are especially beneficial to the growth of the plant. They can provide nutrition for the plant growth [9]. Drug, fertilizer released slowly, providing ample nutrition and medicine protection for seed and seedling in the certain growth period. Plant growth regulator and fertilizer contained in the seed coating agent can promote seed and seedling to grow healthily, raising seed germination rate, increasing seedling quality, enhancing seedling anti-adversity, at last having react on keeping and increasing yield.

Conclusion

A novel environmentally friendly cotton seed coating agent was made by using the natural polysaccharide as the main ingredient, and complemented by fertilizer and trace elements. In the process of preparation of the seed coating agent, the best effect of increasing yield can be obtained when the pH value was 6 and the concentration of natural polysaccharide was 1%. Comparing with the traditional cotton seed coating agent, the yield was enhanced by 9.47%, while its cost was decreased by 35% and it was much safer and more environmentally friendly. And it used enhancing immune function and the antifeeding method to control pests, comparing to the traditional cuke seed coating agent. So it has obvious economic and environmental benefits, having an important expansion application value throughout the world.

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