

# Preparation and mechanism analyses of a new corn seed coating agent

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## ABSTRACT

Due to the severity of the disease and yield losses of corn, this study had achieved a novel corn seed coating agent which was prepared with polysaccharide, fertilizer, microelement and other assistants as raw materials. The dilution and the pH value of the corn seed coating agent, were studied. And the biological toxicity and potential hazards of the agent to environmental protection were assessed. Over the whole trial, the best formula and optimum conditions of a new corn seed coating agent were determined. Also results indicated that compared with the traditional one Jinong No. 4, yields of corn was increased over 9.5%, but cost was reduced over 18.75%. To sum up, this agent self-made had three primary characteristics of high yield, less cost and friendly environment. So it had an important value of application and promotion in main production zone of corn and can bring us obvious economic and environmental benefits.

**Keywords:** Coating; Germination Rate; Inhibitory Rate; Antifeedant Rate; High Yield; Friendly Environment

## 1. INTRODUCTION

Corn is one of the major crops in China, but in recent years, the soil-borne of corn has been aggravated year by year, and corn yields decreased obviously. So the soil-borne of corn has become one of the main factors of corn yields and quality [1,2]. To protect corn from underground pest, provide the source of fertilizer, and increase yields of corn, seed-coating is an effective technology and has been widely applied for crop protection [3]. Studies have shown that a seed coating is effective in preventing and controlling mould-induced diseases and pests causing them, promoting seedling growth, and increasing yields [4,5].

Since the seed coating agent for rain-fed crops first in the 1930s, the research and development of the seed coating agent has been rapid during the past two decades and has provided an economical approach to seed enhancement, especially for larger seeded agronomic and crops [6-8]. However, the conventional toxic seed coating agent currently used such as carbofuran and thiram unavoidably brought serious harm to the environment and human and make the development of ecoagricultural practices impossible [9-11]. Studies showed that only 7 milligrams of carbofuran can kill a person. In addition, it leaves a Long-term pollution, eliminated difficultly from the environment, and becomes a great hidden danger in China's ecological agriculture [12,13].

The dire threat of current conventional toxic seed coating agent forces to seek for a safe and environmentally friendly corn seed-coating agent to resolve the current agricultural and environmental issue. After years of research, the novel seed coating agent (short for LSCA) instead of conventional toxic fungicide, was achieved through advanced biological chelating technology, which prepared with natural polysaccharide as the main material and high-activity plant growth regulator as the auxiliary material. Compared with a conventional seed coating (Jinong No. 4), the effect of LSCA on the resistance to *smut fungus*, seed germination, and corn yield, as well as its toxicity was investigated. Yields of corn was increased over 9.5%, but cost was reduced over 18.75%. With the application of the new coating agent, corn seed-coating agent has better quality and properties such as high yield, low cost, friendly environment, and would have significant economic and environmental benefits.

## 2. MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1. Major Apparatus and Glassware

The major instruments and glassware used in present study were electron constant speed mixer GS28B (Shang-

hai Anting Electronic Instruments Plant, Shanghai, China), high pressure steam sterilizer YXQ-SG46-48SA (Shanghai Boyi Industry Co. Ltd., Shanghai, China), constant temperature and humidity incubator WS-01 (Hubei Huangshi Hengfeng Medical Instrument Co., Ltd., Hubei, China), electronic balance (FA2004, Shanghai Yuefeng Instrument Appearance, Ltd., Shanghai, China), warm up hygrometer STH950 (Summit, USA), biological microscope BX41-12HO2 (Olympus Corp., Japan) and petri dishes (90 cm dia., Shanghai Yuejin Medical Treatment Instrument Plant, Shanghai, China). 24 Orifices of plate.

### 2.1.2. Major Chemicals and Reagents

The major chemicals and reagents used in present study were polysaccharide (PC, self-made in lab), film-forming agent, polyethylene glyco (self-made in lab), the agriculture regulates, acetic acid, carbamide, natrium hydroxydatum, violet pigment (Guangdong shantou Ming frideric food additive Co., LTD), sand, The conventional corn seed coating agent Jinong No. 4<sup>®</sup> (Hubei Provincial Seed Group Co., Ltd., China) (Prefecture Seed Science and Technology Development, Ltd., Henan, China), Artificial feed, *Smut fungus* (North Coating Agents Factory, Tianjin, China), Corn seeds (China Jilin Seed Co.).

### 2.1.3. Experimental Animals

Cutworm (the consistent growth) (provided by Heilongjiang Agriculture University, Sprague-Dawley rats (20 males, 20 females; 200 ± 12 g) (Animal Testing Center of Tongji Medical College of Huazhong University of Science and Technology).

## 2.2. Experiment Methods

The experiment stepwise development and technique route of corn seed coating agent was showed in the **Figure 1**. According to three principles of cheapness, environmental protection, high efficiency, a number of the

recipes of the corn seed coating agents were designed to prevent seedling and increasing yields. Once all the major performance parameters of agents made in lab exceed those of the traditional one Jinong No. 4, we optimized further on the basis of experiment to get the best recipe, then carried the field text, to determine the best formula of agent with a high cost performance and environmental friendship.

### 2.2.1. Comparative Experiment of the Corn Seed Coating Agent in the Laboratory

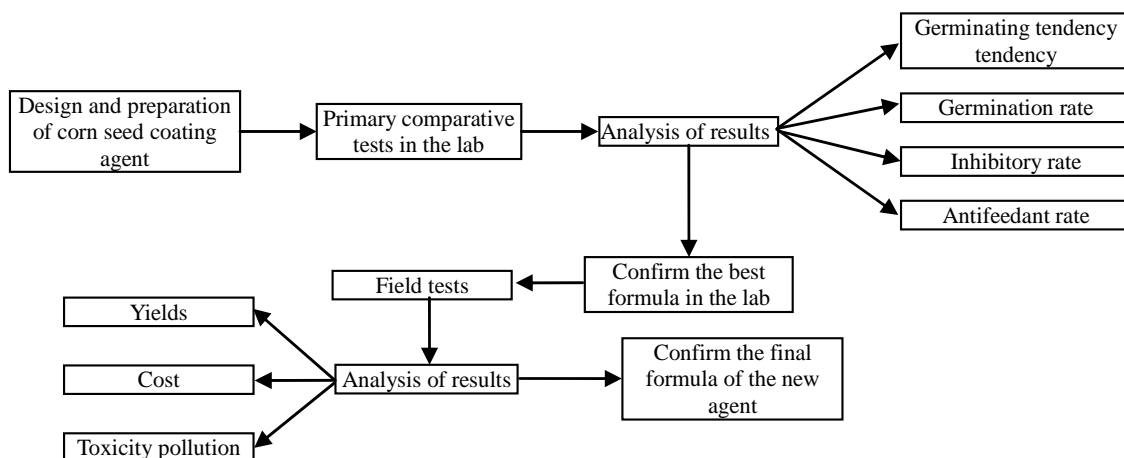
As stated by the manufacturer of Seafresh Chitosan (Lab) Co. Ltd., China, the natural polysaccharide with a molecular weight of from 20 to 1500 KD and deacetylation degree of from 80% to 90%, was prepared as: 1 wt% acetic acid to dissolve polysaccharide completely to a final concentration 1wt% under stirring at 25°C for approximately 4 h.

The optimal coating formulation was determined through orthogonal test. Aqueous solutions of fertilizer, microelement, ethylene glycol, film former and crocuses red were prepared respectively at a certain concentration. After mixing every component completely at room temperature.

Concrete experimental process are as follows:

### 2.2.2. Test of Seed Germination

Comparative experiment of the corn seed coating agent in the laboratory was as follows: the same corn seeds were coated with the new seed coating agents (A-E) and the conventional one (Jinong No. 4) respectively, and then put in the constant temperature and humidity incubator under the same condition of the constant temperature 28°C ± 1°C and the constant humidity 85%. Experimental results of the germination tendency and germination rate should be observed and compared every day. Each treatment was replicated 3 times.



**Figure 1.** The experiment stepwise development and technique route of corn seed coating agent.

According to the seed test procedures methods of international seed association (ISTA) [14], germinating tendency (GT) was calculated on the 4th day and germination rate (GR) was calculated on the 7th day. The calculation formulas were as follow:

$$GT (\%) = (G_3/S) \times 100 \quad (1)$$

$$GR (\%) = (G_7/S) \times 100 \quad (2)$$

where  $G_3$  is the number of germinated seeds on the third day;  $G_7$  is the number of germinated seeds on the seventh day;  $S$  is the number of total seeds investigated.

### 2.2.3. Antibacterial Experiment in the Lab

According to microbiological test requirements for inhibitory rate trial, the *Setosphaeria turcica* blocked 5 mm were inoculated to agar which was sterilized with a high-pressure steam and mixed with different agents, water as to control respectively, and then cultivated at 25°C for 2 days [15]. The tests were repeated 3 times and recorded the diameter using cross measuring method. The following formula was used to calculate the inhibitory rate of corn seed coating agents.

$$IR (\%) = [(D_0 - D)/D_0] \times 100 \quad (3)$$

where IR is Inhibitory rate;  $D_0$  is colonies diameter in the control petri dish, and  $D$  is the colonies diameter in the test petri dish.

Once all the major performance parameters of agents made in lab exceed those of the traditional one, then we optimized further on the basis of experiment to get the best recipe, named after LSCA, repeated the above and stability experiment.

### 2.2.4. Antifeedant Test of Pests in the Lab

According to the guideline for laboratory bioassay of pesticides, the antifeeding effect of LSCA was studied with the artificial mixed feeding method. The process of the antifeeding test was as follows: a certain amount of artificial feed mixed a certain amount of LSCA, the proportion (w/w) were 5%, 10%, 15%, and an untreated group (CK) as control was designed. Then the feeds above and the consistent growth cutworms were put into the Orifices plates. Cutworms were feeded in the incubator at a temperature of 25°C ± 1°C, and relative humidity of 75% - 85%. After 48 h the remaining feeds (control and treated) were weighted, the test was valid only the mortality of larvae within 5%. Every text replicated three times the formula is as follows [16]:

$$AR (\%) = [(W_0 - W)/W_0] \times 100 \quad (4)$$

where AR is Antifeedant rate;  $W_0$  is the weight of control feed and  $W$  is the weight of feed treated by corn seed coating agent.

### 2.2.5. The Security Test

According to the toxicological test methods of pesticides for registration, the toxic effect of LSCA and Jing No. 4 were studied with the LD<sub>50</sub> (the median lethal dose) method. In the study, the rats were fasted overnight, then treated at various dosages of LSCA and Jinong No. 4, by oral ingestion and skin infection respectively. The toxic symptoms of rats and the median lethal dose (LD<sub>50</sub>) were recorded after 14 days. According to the results of the toxicity test, the difference of toxicity LSCA and Jinong No. 4 was estimated.

### 2.2.6. Field Trials

Through the above formula and the experimental results, we calculated the germinating tendency and germination rate, inhibitory rate, antifeedant rate etc., and finally the best formulation was evaluated in the field.

Field trial was carried out to verify further whether the efficacy of (LSCA) in the laboratory was stabilized, and the effect on the yield was higher than that of the traditional one. In this test, the method of seed treatment was the same as that in the laboratory experiment, the corn seeds were coated with LSCA and Jinong No. 4 in the proportion of 1:50 (w/w). The experiment was conducted at Hubei Provincial Seed Group Company. The experiments were designed as a randomized block, and each treatment was replicated three times.

### 2.2.7. Statistic Analysis

Analyses of variance (ANOVA) and Duncan means comparison test with significance level of 0.05 were applied.

## 3. RESULTS AND DISCUSSION

### 3.1. Results

#### 3.1.1. Primary Experiment Results in the Laboratory

The primary purpose was to select the best environmentally friendly corn seed coating agent, the price-performance ratio of which preponderated over that of Jinong No. 4. Primary experiment results in the laboratory were showed in **Table 1**, corn seed coating agents A-H were prepared-self, the traditional one Jinong No. 4, CK uncoated seed as control.

It can be seen from **Table 1**, different agents had different influences on corn seedling growth, and the main indexes of B-E exceeded over Jinong No. 4. It can be seen that E had a most effect on germinating tendency, germination rate, inhibitory rate, and enhanced by 9.4%, 4.0%, 8.1% respectively compared to Jinong No. 4. E had a positive promotional effect on seed germination

and seedling growth. So E was the best formulation, named after LSCA.

It can be seen from **Table 2**, these results were stabilized and consistent with those in **Table 1**.

These results showed that seed coating agents played a significant role in improving germinating tendency, germination rate and inhibitory rate, but the efficiency of LSCA was obviously superior to Jinong No. 4.

### 3.1.2. Optimization Experiments in the Laboratory

Based on the best formula of the seed coating agent LSCA, changing two main factors such as pH and the dilution to confirm the optimum conditions of seed coating agent, so that the new agent can achieve the best effect and improve the price-performance ratio.

### 3.1.3. Influence of pH Value of Seed Coating Agent

Influence of pH value (the range of 4.0 - 7.0) of seed coating agent was shown in **Table 3**.

As can be seen in **Table 3**, the best effects of germination tendency, germination rate and inhibitory rate were observed at pH 6.0. From 4.0 to 6.0, the higher the pH value was, the higher main parameters, and they reached the maximum at pH 6.0. When pH was above 6.0, with the increasing of the pH value, they declined. Therefore the best pH value of corn seed coating agent was 6.0.

**Table 1.** The preliminary experimental results of the seed coating agents in the laboratory.

Treatments	GT (%)	GR (%)	IR (%)
A	72.5c	86.4b	80.3c
B	77.2b	89.8b	83.7c
C	80.2a	92.4a	86.8b
D	83.6a	94.3a	89.5b
E	85.3a	93.6a	92.3a
F	80.5a	92.7a	91.2a
G	77.4b	90.1a	88.5b
H	75.6bc	88.4b	85.8b
Jinong No. 4	76.9b	88.7b	84.2c
CK	68.5d	76.4c	0

Averages in column separated by Duncan's new multiple range test, 5% level. A-H: seed coating agents self-made; Jinong No. 4: a conventional seed coating agent; CK: blank control. GT: Germinating tendency; GR: Germination rate; IR: Inhibitory rate.

**Table 2.** The preliminary experimental results of the seed coating agents in the laboratory.

Treatments	GT (%)	GR (%)	IR (%)
LSCA	88.6b	94.8a	93.6a
Jinong No.4	79.5d	87.1b	85.6bc
CK	78.6d	85.5bc	-

These results are consistent with those reported by Jun-ang [17] that the optimal pH range was between 5.3 and 6.0 for the highest antifungal activity. This increase in pH was due to the increased consumption of H<sup>+</sup> ions in solution by the protonation of the free amine groups of polysaccharide [18]. When the pH value was 6.0, the protective film on 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 6.0, the film had better permeability which can guarantee plants supplying ample oxygen and water, and creating a beneficial condition for the better growth of seeding.

### 3.1.4. Influence of the Dilution of Seed Coating Agent

Results of the experiment in the lab showed different proportion of agent and seeds had different influence on corn seedling growth in **Table 4**.

It can be seen from **Table 4**, the effect on sprout potential, germination rate of 1:30, 1:40 was lower than that of CK, especially at 1:30, the agent restrained the growth of seedlings, showing obviously a series of signs of hide-bound symptoms such as shorter seedling and stem thin. When at 1:50, 1:60, 1:70, the main parameters of LSCA was higher 3 times than that of CK and the growing and leaf color of seedlings were consistent. Therefore, 1:50 was the best proportion. When at 1:50, the germination

**Table 3.** The influence of pH on the main performance parameters of seed coating agent.

pH	GT (%)	GR (%)	IR (%)
4.0	74.5d	82.5c	83.6c
4.5	78.9d	86.5b	86.3b
5.0	82.6c	89.9b	88.5b
5.5	86.6b	91.7a	91.5a
6.0	88.6b	93.7a	93.6a
6.5	85.6bc	91.4a	92.9a
7.0	82.6c	90.6a	90.2a

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

**Table 4.** Compare of germination energy and germination rate in different proportion of seed.

The dilution of LSCA	GT (%)	GR (%)	IR (%)
1:30	84.9c	89.9b	89.5b
1:40	86.6b	91.7a	91.3a
1:50	88.6b	94.8a	92.9a
1:60	87.5b	92.6a	90.5a
1:70	85.6bc	90.6ab	87.6b
CK	82.6c	86.5b	-

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

rate was up to 94.8%, which displayed obviously to promote sprout and growth of seedlings. It can be explained that when the proportion was 1:50, the protective film provides the favorable conditions for seed germination, growth, and plays significant role in maintaining bactericidal and insecticidal effect.

### 3.1.5. Antifeedant Effect of Seed Coatings

Results of antifeeding test showed that LSCA had an excellent antifeedant effect on selective antifeedant rate by observing experimental process and analysing of weight. According to the observation, the artificial feed containing LSCA was rarely bitten, while the artificial feed without LSCA (CK) was nearly eaten up. From **Table 5**, it showed when the proportion of LSCA was 15%, 10%, 5% on cutworm, the antifeedant rates were respectively 87.4%, 70.9% and 48.3%. All of those indicated the antifeedant effect of LSCA was increased with increasing dosage of LSCA.

### 3.1.6. Results of Acute Toxicity Test

Results of acute toxicity test showed there were significant differences in the median lethal dose ( $LD_{50}$ ) between LSCA and Jinong No. 4 (**Table 6**).

In the acute oral toxicity test, rats infected with a high dosage of Jinong No. 4 exhibited toxic symptoms after 3 - 6 min such as systemic muscle spasm, salivation, and even convulsions, and sticky nasal and ocular secretions

in the 12 hours before death. The rats infected with low dosages still showed a sign of slight muscle spasm. However, the rats infected with LSCA didn't exhibit the previously mentioned symptoms and exposed to a low level. The datas showed that the acute oral  $LD_{50}$  and acute dermal  $LD_{50}$  in female rats were  $607 \text{ mg}\cdot\text{kg}^{-1}$  and  $2106 \text{ mg}\cdot\text{kg}^{-1}$ , respectively. In the acute skin toxicity test, the rats were observed consecutively for two weeks when they were infected with the toxicant. By safety experiment, we see that the acute toxicity of Jinong No. 4 was significantly higher (about 2 times) than that of LSCA. Therefore, the novel seed-coating agent LSCA was safe for human and meets the requirements of the environment protection during usage and disposal.

### 3.1.7. The Comparative Result of Field Trials

The comparative result was shown in **Table 7**.

Results of field trial showed that LSCA noticeably improved obviously the main performance indices such as the germination index, seedling rate (**Table 7**), when comparing with Jinong No. 4, the germination rate and per ha yield of LSCA were increased by 5.6% and 9.5% respectively; when compared to the uncoated corn seed (CK), and they were enhanced by 6.0% and 13.6% respectively, while the cost was declined by 18.75%. It had indicated that the germination energy and germination rate in the laboratory were consistent with the trend of increasing yield in field experiment.

**Table 5.** The experimental result of antifeedant effect of the seed coating agents.

Treatment	Dosage of LSCA (%)	Weight of AF (g)	Weight of AF in 48 h (g)	Feeding volume (g)	AF (%)
LSCA	15	5.488	5.193	0.294	87.4a
	10	5.693	4.966	0.677	70.9b
	5	6.267	4.963	1.204	48.3c
CK	-	5.550	3.213	2.327	-

Averages in column separated by Duncan's new multiple range test, 5% level. AF means artificial feed; LSCA means the novel seed coating agent; CK means the blank control.

**Table 6.** The result of the security test in rats.

Experimental Groups	Results of acute toxicity test			
	Aot $LD_{50}/\text{mg}\cdot\text{kg}^{-1}$	TC	Ast $LD_{50}/\text{mg}\cdot\text{kg}^{-1}$	TC
LSCA	798 (Male)	Slight	2239 (Male)	Slight
	607 (Female)	Slight	2106 (Female)	Slight
Jinong No.4	243 (Male)	Moderate	1792 (Male)	Moderate
	127 (Female)	Moderate	1554 (Female)	Moderate

Acute oral toxicity grading scale: 1) Slightly toxic:  $LD_{50} > 500 \text{ mg}\cdot\text{kg}^{-1}$ ; 2) Moderately toxic:  $50 < LD_{50} < 500 \text{ mg}\cdot\text{kg}^{-1}$ ; 3) Highly toxic:  $LD_{50} < 50 \text{ mg}\cdot\text{kg}^{-1}$ . Acute dermal toxicity grading scale: 1) Slightly toxic:  $LD_{50} > 2000 \text{ mg}\cdot\text{kg}^{-1}$ ; 2) Moderately toxic:  $200 < LD_{50} < 2000 \text{ mg}\cdot\text{kg}^{-1}$ ; 3) Highly toxic:  $LD_{50} < 200 \text{ mg}\cdot\text{kg}^{-1}$ .

**Table 7.** The investigation results of the field trial.

Measurement Indicators	LSCA	Jinong No. 4	CK
Germination index	11.2	10.8	10.2
Seedling rate (%)	96.4	92.5	91.9.
The length of function leaf (cm)	96	84	83
The thick of stem (cm)	3.6	2.7	2.5
The rate of infected plant (%)	0	5.3	25.6
Yield (kg·ha <sup>-1</sup> )	986	892	852
Cost (US \$ ·kg <sup>-1</sup> )	1.3	1.6	
Toxicity	Slight	Moderate	

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

## 3.2. Discussion

### 3.2.1. The Mechanism of Insect Prevention

The most important difference between LSCA and Jinong No. 4 was LSCA used PC as the main ingredient, which can improve plant pest and disease resistance capability, and promote plant's growth, crop yield and quality [19,20]. When the plant is attacked by pest, PC as an inducer stimulates plants to produce a series of defense reactions and produce secondary structure (e.g. periderm, corpus callosum, glue sluggish body, invade fill body, excise, halo, etc.) and some functional molecules (e.g. plants defend element, phenolic compounds, duration of related proteins, pathogens, enzyme inhibitors, etc.), which are inexistent before stimulating. These substances directly or indirectly suppress chemical sensors of insect mouthpart, and block the nerve conduction, make its information transfer of feeding behavior interrupted, or causing abnormal discharge from animals to get correct taste of information and not to eat [21-24]. So LSCA controls pests through the approach of repelling pests and enhancing the immunity of seeds, instead of poisoning pests. Finally pest lose taste function and refuse feeding reaction.

In addition, if PC is used in advance, plants will mistake pest coming so that they can produce resistant material (phenylalanine solution ammonia enzyme (PAL), peroxidase (PO) and polyphenol oxidase (PPO), phytoalexin etc, protein related to the disease such as chitinase, and beta arene glucan enzyme etc.), and thus act the genes of plant resistance and make plant produce system antibody, which can keep a high level for a long time to repel pests [25-27]. PC as an inducer using in the earlier stage crop growth is better than used in the later period.

The inducible resistance function of PC is like to hit the precaution for plants, which makes plants less ill or no, thus promote plant's growth, crop yield and quality.

### 3.2.2. The Mechanism of Yield Increase

The field experiment results showed that seeds coated with PC had better resistance to *smut fungus* and higher germination percentage and higher crop yields than the control. The first main reason is the mentioned-above mechanism of insect prevention, and another is that PC can enhance the resistance against *smut fungus* and has a repellent effect to the pests in the soil, so that the plants are safe from threats and damage by disease and have a positive seedling growth and the fruits of harvest [28-30].

The second reason contributes to PC of LSCA, which has the ability of forming a dense protective film on the surface layer of seeds. The film increases the brightness of the coated seeds. In this case, the fungicide was glued on the surface of the seeds, it is an advantage, since it allows the continuous contact of the fungicide with the seed surface and increased the resistance of the fungicide to be removed by the wind or rain. Thus, the agent was acting not only as antimicrobial coating itself but also as a carrier system of agrochemicals [31,32].

The film has a good selective permeability which can prevent oxygen from entering the film, restrict loss of CO<sub>2</sub>, and maintain a high concentration of CO<sub>2</sub> in the film [33-35], so as to restrain the seed's respiration, maintain the seed moisture and absorb the soil moisture, and cut off excessive soil moisture to prevent the seed from corrupting. In short the film can provide a good growth environment for seeds germination and growth and make the internal nutrient consumption of seeds fall to the lowest possible level [36-38]. It is just that this layer of natural polymer membranes plays a vital role on corn yield and antifeedant effect.

The third reason contributes to the corn yield increase may be the effect of the trace elements and fertilizer contained in LSCA. The trace fertilizer can increase the activity of an enzyme and provide abundant nutrient sources for germ growing.

## 4. CONCLUSIONS

The formula was as follows: 1% PC 56%, sodium hydroxide 5%, trace fertilizers 22%, trace element 1.5%, active ingredients 1%, filmogen 4%, plant growth regulator 2%, water 6.5%, natural pigment 2%. In the process of preparation, the pH of the agent was adjusted to 6.0, and the dilution was 1:50. The results showed that a novel formulation had increased the yield by about 13.6%, 9.5% over that of CK and Jinong No. 4, respectively. Furthermore, the cost of LSCA was 18.75% less and the toxicity was lower than that of Jinong No. 4. So it is safe, non-polluting, and has significant economic and environmental benefits.

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