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Preparation and application of a novel environmentally friendly cucumber seed coating agent

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A novel cucumber seed coating agent was made from natural polysaccharide, fertilizer and microelement, etc., which achieved a good effect on increasing cucumber yield and antifeeding against pests. The foremost difference between this seed coating agent and conventional ones was that it controlled pests through the approach of repelling pests and enhancing the immunity of seeds, but not by killing pests. Results indicated that this seed coating agent had excellent control of pests and diseases and increased yield by 8.4 to 10.8%, while the material cost was decreased by 16.7% compared with the conventional toxic seed coating agent. In addition, results showed it was safe for man and livestock without causing any pollution and harm. Therefore it had three characteristics of high yield, less cost and friendly environment.

Key words: Environmentally friendly type, cucumber seed coating agent, polysaccharide, antifeedant, germination percentage.

INTRODUCTION

Cucumber is one of the main thermophilic vegetables, its planting area accounts for more than 15% of the total area planted to vegetables in China. Now the biggest problem is chilling injury and pests damaged in seedling stage, yet seed coating is the main method and key technology to control of pests and diseases (Xiong, et al 2004). Seed coating technology has developed rapidly during the past two decades and has provided an economical approach to seed enhancement, especially for larger seeded agronomic and horticultural crops (TeKrony, 2006). 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 (Gesch and Archer, 2005). But the conventional toxic seed coating agent unavoidably brought harm to the environment and man during usage and disposal. In addition, it leaves a persistent pollution that has been difficult to eliminate from the environment for many years, and it has become a great hidden danger in China's ecological agriculture (Raveton et al., 2007). So we prepared a novel environmentally friendly cucumber seed coating agent which was non-toxic, non-polluting and achieved a good effect on increasing cucumber yield and had the excellent effect of antifeedant to pests. In addition, it was safe for man and livestock without causing any pollution and harm.

The novel seed coating agent (short for NSCA) was prepared through advanced biological chelating technology, with natural polysaccharide as the main material and high-activity plant growth regulator as the auxiliary material (Herbert, 2003; Qiu, et al, 2005). It could enhance plants disease-resistant ability and had strong antifungal bioactivity; in addition, it has special odor which can generate excellent antifeeding role to pests. With the application of the new coating agent, cucumber seeds had better quality and properties that resulted in an increased yield, showing a great market prospect in the major wheat producing areas.

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MATERIALS AND METHODS

Main apparatus and reagents

The reagents used include natural polysaccharide (prepared in laboratory), sodium hydroxide, acetic acid, borax, fertilizer, ethylene glycol, microelement, film former, penetrating agent (analytically pure, Hubei University Chemical Plant), violet pigment (Guangdong Shantou food additive of Mingde Co., Ltd.), conventional seed coating agent (2.5% Shileshi suspension concentrates seed coating agent, Switzerland), rats (Animal Testing Center of Tongji Medical College of Huazhong University of Science and Technology), *Sphaerotheca fuliginea* and *Fusarium oxysporium* (College of Plant Science and Technology, Huazhong Agricultural University) and new cucumber seeds (Jingyou number two, Wuhan seed Co., Ltd of Tianhong in China). The apparatus used includes constant temperature and humidity incubator (Model No.WS-01, Hubei Huang Shi Hengfeng Medical Inaszdxftrument Co. Ltd.), warm up hygrometer (Model No.STH950, SUMMIT, USA), and high pressure steam sterilizer (Model No.YXQ-SG46-48SA, Shanghai Boxun Industry Co. Ltd.). The major instruments and glassware used in present study were electronic balance (Model No.FA2004, Shanghai Yuefeng Instrument Appearance Ltd.), electron constant speed mixer (Model No.GS28B, Shanghai Anting Electronic Instruments Plant), and Petri dishes (90 cm diameter, Shanghai Yuejin Medical Treatment Instrument Plant).

Preparation of NSCA

The natural polysaccharide was prepared in 1wt% acetic acid to a final concentration of 1wt% and stirred at room temperature for approximately 3 h. The components have a molecular weight of from 30 to 1400 KD, deacetylation degree of from 80 to 90%.The optimal formulation of the novel seed coating agent (NSCA) was determined through orthogonal test. NSCA was prepared with the following components (wt%): Natural polysaccharide 55, fertilizer 15 , microelement 15, sodium hydroxide 4,ethylene glycol 2, borax 2, film former 1, penetrating agent 0.5, violet pigment 0.5, and water 5. Aqueous solutions of fertilizer, microelement, sodium hydroxide, ethylene glycol, borax, film former, penetrating agent and violet pigment were prepared respectively at a certain concentration. After mixing the entire component completely at room temperature, the working liquid of NSCA was a purple suspension.

Laboratory method for antifeeding test

According to the guideline for laboratory bioassay of pesticides, the antifeeding effect of NSCA was studied with the artificial mixed feeding method. The treatments were divided into four groups: Every 100 g of artificial feed containing 13.3, 10 or 8 mg of NSCA, and uncoated cucumber seeds (CK) to serve as control. Then the feeds as previously mentioned were put into each hole with diameter of 1.5 cm and depth of 1.5 cm. The consistent growth insects were chosen and placed side the hole and each treatment were replicated 3 times. A layer of blow moulding plate was placed between the pest-feeding plate and the cover to retain moisture and to prevent the tested pests from escaping. The antifeeding effect was determined at 48 h after cultivation in the incubator at a temperature of 25±1℃, and relative humidity of 75 to 85%, and then weights the artificial mixed feeds. The selective antifeedant rate was calculated by the following formula (1):

$$\text{Antifeedant rate}(\%) = \frac{\text{A-B}}{\text{A}} \times 100\% \quad (1)$$

where A is the weight of the control group, and B is the weight of the treatment group.

Laboratory method for year-on-year test

The year-on-year test in laboratory was done to determine the enhancement effect of NSCA through comparing the germinability and germination percentage of NSCA, CA and CK. The cucumber seeds were coated in the proportion of 1:40 (w/w) and then dried by airing at room temperature for 20 to 30 min to prepare for use. Uncoated cucumber seeds (CK) were prepared as blank control group.

Laboratory method for the germination test

According to the rules for seed testing of International Seed Testing Association (Wu et al., 2003),100 seeds taken from each group were arranged on two layers of wet filter paper in each Petri dish filled with wet sand. Each Petri dish contained 25 seeds and each treatment replicated 3 times. All Petri dishes were incubated in the constant temperature and humidity incubator at 28±1℃ and air relative humidity of 85%. The germinability (GE) and germination percentage (GP) of cucumber seeds were investigated on the third day and seventh day, respectively. The calculation formulas were as follows:

$$GE = \frac{C}{E} \times 100\% \quad (2)$$

$$GP = \frac{D}{E} \times 100\% \quad (3)$$

where C is the number of germinated seeds on the third day, D is the number of germinated seeds on the seventh day, and E is the number of total seeds investigated.

Laboratory method for fungistatic text

According to microbiological test requirements for inhibitory rate experiment, by measuring the growth rate we evaluated the two seed coating agents of antifungal activity on mycelial growth in two phytopathogens including *Sphaerotheca fuliginea*, and *Fusarium oxysporium* on PDA; cultivated the phytopathogen for 2 days and plugged with cork borer to form a cake of 5 mm in diameter, then, inoculated the cake in another PDA medium containing seed coating agent and incubated at 25℃ for 7 days. The fungistatic effect was observed under the biomicroscope and the diameter of colony was measured twice. The average value was recorded. Each treatment was replicated three times. The inhibitory rate was calculated by the following formula (Sun et al., 2004):

$$Inhibitory\ \ rate = \frac{F-G}{F} \times 100\% \quad (4)$$

F is the colony diameter of the control group and G is the colony diameter of the treatment group.

Through the Formula (4) and the experimental results, we calculated the germinability, germination percentage, inhibitory rate, etc., and finally determined the optimal formula of NSCA through the result of field trial.

Laboratory method for toxicity test

According to the toxicological test methods of pesticides for registration (The Chinese State Standard GB15670-1995), the toxic effect of NCSA and CA were studied with the LD_{50} method. There were two major exposure routes by which toxicity materials may enter the body: Ingestion (gastrointestinal tract) and dermal contact.

Table 1. The comparison results of antifeeding test.

Treatment	Dosage of NSCA (mg)	Repetition	Weight of AF (g)	Weight of AF in 48 h (g)	Feeding volume (g)	Antifeedant rate (%)
NSCA	13.3	1	5.25a	4.77c	0.48a	78.76a
		2	6.92b	6.55a	0.37d	83.63b
		3	5.49a	5.08b	0.41a	81.86a
		Average				81.42a
	10	1	5.45c	4.73a	0.72b	68.14b
		2	5.84a	5.06b	0.78a	65.49c
		3	6.31c	5.44b	0.87a	61.50a
		Average				65.04a
	8	1	6.35b	5.30a	1.05d	53.54c
		2	6.03d	4.81b	1.22a	46.02a
		3	5.62a	4.53c	1.09a	51.77b
		Average				50.44a
CK	-	1	5.36a	3.15a	2.21a	-
		2	6.23b	4.00b	2.23b	-
		3	6.55c	4.21d	2.34b	-
		Average	6.05a	3.79b	2.26b	-

Values within a column followed by different letters are significantly different (p =0.05) according to the Duncan's multiple range test. AF means artificial feed; NSCA means novel seed coating agent; CA means conventional seed coating agent; CK means blank control.

The rats were fasted overnight before treatment, 14 days later, the toxic symptoms of rats were observed and the median lethal dose (LD_{50}) was determined after infection with seed coating agent at various dosages. According to the results of the toxicity test, we determined the differences in the aspects of toxicity and safety, etc. between NSCA and CA.

Field trial

The field trial was conducted in the plots of the experimental field in Hubei Provincial Seed Group Co., Ltd, China, in 2008 and 2009. In this test, the method of seed treatment was the same as that in the laboratory experiment. The cucumber seeds were coated with each of the seed coating agents in the proportion of 1:40 (w/w) and then dried by airing at room temperature. After spreading and airing for about 30 min, the seeds were sown into the field. The experiments were designed as a randomized block design with each treatment consisting of several plots separated from each other by a row 20 cm wide.

Each plot (4.0 m wide by 5.0 m long) contained 500 cucumber seeds. Each plot was sown either with coated seeds or with uncoated seeds to serve as control and field management was the same for all experimental plots. The emergence situation 7 days after sowing was examined, the growth situation in growth period investigated and whether the maturation delay before harvest was observed. Total yield for each plot was determined by weighing the 10 cucumber selected random and then investigating the yield of per plant. The best formulation was selected on the basis of yield performance and cost effectiveness.

RESULTS

Antifeedant effect of NSCA

Results of antifeeding test showed that the antifeedant effect of NSCA was increased with increasing amount of dosage in every 100 g of artificial feed. The antifeedant rate of containing 13.3 mg was best in antifeedant effect (Table 1 and Figure 1). Figure 1 showed excellent antifeedant effect by an analysis of weight and selective antifeedant rate of NSCA; the artificial feed without adding NSCA was nearly eaten up while the artificial feed containing NSCA was rarely bitten. It is found that the antifeedant rate of every 100 g of artificial feed containing NSCA 13.3, 10 and 8 mg on cutworm was 81.42, 65.04 and 50.44%. Compared with CK, the antifeedant effects of NSCA were significantly better than those of CA.

Effect of NSCA on seed germination

Results of year-on-year test showed different seed coating agents had different influence on cucumber seedling growth (Table 2). The average level of germinability of seeds coated with NSCA was 86.05%, with a range from 84.8 to 87.6%, about 8.75% higher

Figure 1. Antifeeding effect of NSCA and CK on cutworm in 48.

Table 2. The comparison results of year-on-year test in the laboratory.

Compare index	Treatment					
	NSCA1	NSCA2	NSCA3	NSCA4	CA	CK
GE/%	84.8a	86.3c	87.6a	85.5b	77.3b	70.5a
GP/%	91.6b	88.6a	92.5b	93.2a	84.9a	79.7d
inhibitory rate/%	89.4a	88.4b	93.5a	95.4a	85.5c	-

Value within a column followed by different letters are significant different (p=0.05) according to Duncan multiple range test. GE means germination energy; GP means germination percentage; NSCA means novel seed coating agent; CA means conventional.

than that of seeds coated with CA and almost 15.55% higher than that of CK. The average level of seeds coated with NSCA showed 6.58 and 11.78% increase in germination percentage compared with CA-treated seeds and CK, respectively. Similar results were recorded with respect to germinability and germination percentage. The average level of inhibitory rate of seeds coated with NSCA was 91.68%, about 6.18% higher than that of seeds coated with CA .The results showed that four types of NSCA were superior over CA and CK in terms of germinability, germination percentage and inhibitory rate and grew well.

Results of acute toxicity test

Results of acute toxicity test showed there were significant differences in the LD_{50} between these two seed coating agents (Table 3). In the acute oral toxicity test, rats infected with a high dosage of CA would exhibit toxic symptoms after 3 to 6 min such as systemic muscle spasm, salivation, and even convulsions, and sticky nasal and ocular secretions in the 12 h before death. The rats

Table 3. The comparison results of acute toxicity test.

Treatment	Rats' gender	Acute oral toxicity LD_{50} ($mgkg^{-1}$)	Acute skin toxicity LD_{50}($mgkg^{-1}$)	Toxicity classification
NSCA1	Male	843	2873	Low-toxic
	Female	754	2775	Low-toxic
NSCA 3	Male	863	2646	Low-toxic
	Female	764	2745	Low-toxic
NSCA 4	Male	872	2887	Low-toxic
	Female	787	2798	Low-toxic
CA	Male	216	763	Moderate-toxic
	Female	192	645	Moderate-toxic

Acute oral toxicity grading scale: Low-toxic: LD_{50} > 500 $mg{\cdot}kg^{-1}$; moderate-toxic: 50 < LD_{50} < 500$mg{\cdot}kg^{-1}$. Acute skin toxicity grading scale: Low-toxic: LD50 > 2000 $mg{\cdot}kg^{-1}$; moderate-toxic: 200 < LD50 < 2000 $mg{\cdot}kg^{-1}$ (Chinese acute toxicity classification standard).

Table 4. The comparison results of field trial.

Treatment	Germination percentage (%)		Average output per plant (kg)		Yield (kg ha^{-1})		Cost (US $ kg^{-1})	Toxicity and pollution
	2008	2009	2008	2009	2008	2009		
NSCA1	90.2a	91.4b	3.4a	3.2c	222100a	209100b	1.5	None
NSCA 3	90.4c	89.3a	3.2b	2.9a	208700b	193950a	1.5	None
NSCA 4	91.6a	91.7b	3.7c	3.4a	242650b	223500a	1.5	None
CA	84.3a	85.6b	3.1d	2.9c	202550b	192750b	1.8	Serious
CK	78.5b	77.8a	2.9b	2.7c	189700b	177900b	-	None

Values within a column followed by different letters are significantly different (p =0.05) according to the Duncan's multiple range test. NSCA means novel seed coating agent; CA means conventional seed coating agent; CK means blank control.

infected with low dosages still showed slight muscle spasm. However, the rats infected with NSCA did not exhibit the toxic symptoms aforementioned. In the acute skin toxicity test, the rats were observed consecutively for two weeks after they were infected with the toxicant according to the conventional method. There is no significant difference between the median lethal dose (LD_{50}) to the male and that to the female rats for NSCA, and the acute toxicity of NSCA in this study is much lower than that of the traditional agent. Therefore, the novel seed-coating agent NSCA is safe for the public and meets the environmental requirements during usage and disposal.

Results of field trial

Results of field trial showed that NSCA noticeably improved the main performance indices such as the germination percentage, the average yield per plant and the yield per ha (Table 4), while the comparative results between NSCA and CA were observed in the plots of the experimental field in Hubei Provincial Seed Group Co., Ltd. It was concluded that NSCA-coated group was the best compared with the other groups for corn output, which showed the increase of 18.3% over CK and 10.8% over the CA-coated group in 2008. In 2009, the output of NSCA-coated group was 17.4% higher than that of CK and 8.4% higher than that of CA-coated group. Furthermore, the cost of NSCA was 16.7% less than that of CA. These results confirmed that seed coating agents can not only significantly improve seed germination but can also enhance cucumber yield. The statistical analysis showed that there was significant difference among the three treatments in cucumber yield. So the ratio of performance to price for NSCA is much higher than that of CA. Correlative analysis proved the main performance indexes in the field experiment had significant positive correlation with the laboratory experiment.

DISCUSSION

The mechanism of antifeedant effect

Because NSCA stimulates plants to produce systematic antibodies, once a certain part of the plant produces resistance it can induce the whole plant to resist pests and diseases, which can produce repellant effects to deter insect pests. As a plant disease resistance exciton, NSCA is also believed to be able to adjust enzymatic activity related to the disease resistance in plants. The perfect combination of polymer polysaccharide and receptor proteins on the plant cell membrane can bring resistant material such as plant antitoxin, polyphenol oxidase, peroxidase, catalase, etc. and antibacterial materials such as salicylic acid and jasmonic in the process of activating disease immunity, which produced obvious antifeedant effect. In addition, the NSCA with natural antifungal property can produce induce systemic resistance and produce significant repellent action to insect pests of rice seed (Shibuya and Minami, 2001; Ma and He, 2001). The restraint of feeding behavior might because information input sensor was interrupted. The odour from NSCA played a barrier function to feeding behavior or directly act on the nervous system of animals which caused the unusual discharge of the nervous system and prevented animals from getting correct information of taste, thus, it can make appropriate feeding reaction.

The mechanism of the yield increase effect

The natural polysaccharide has excellent film-forming property, making it easy to form a semi-permeable film on the seed surface which can maintain the seed moisture and absorb the soil moisture, and thus it can promote seed germination. The natural polysaccharide film is also considered to have a good selective permeability, which can prevent oxygen from entering the film, restrict loss of CO_2 and maintain a high concentration of CO_2 in the film, so as to restrain the seed's respiration, and thus, make the internal nutrient consumption of seeds fall to the lowest possible level (Furbank et al., 2004). Furthermore, a semi-permeable film on the seed surface can delay the release of fertilizer elements and reduce nutrient losses. This kind of semi-permeable film is believed to be able to maintain the seed moisture and absorb the soil moisture, and thus it can promote seed germination (Sigler and Turco, 2002).

Another reason for the cucumber corn yield increase may be the effect of the trace elements and fertilizer contained in NSCA, which can provide adequate nutrition and protection for plant growth. The plant growth regulator and trace fertilizer in NSCA can promote seedling growth, improve the germination rate of seed and quality of seedlings; it plays an increasing grain yield role finally. Also, natural polysaccharide as a novel plant disease inhibitor can induce and improve the disease resistance of plant, thus has a repellent effect to the pests in the soil (Chen and Xu, 2005). The natural polysaccharide possesses the natural antifungal role, which increased the permeability of the outer membrane and inner membrane and ultimately disrupted bacterial cell membranes, with the release of cellular contents (Liu et al., 2004; Nielsen et al., 1994). In addition, the film-forming property itself also hinder the direct contact between pathogens and host tissues or cells, thus effectively reduce the effect of plant diseases and insect pests.

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