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RESEARCH ARTICLE

Preparation and study of an environmentally friendly seed-coating agent for cucumber

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Abstract **Traditional seed-coating agents are widely used, and their accumulative toxicity in soil brings a great hazard to natural environment and human health. In this study, a novel cucumber seed-coating agent was prepared from natural polysaccharide, fertilizer and microelement, etc. Results indicated that the agent had an excellent control effect on pests and increased yield by 8.5% to 9.3%, while the material cost was decreased by 16.7% compared with the traditional toxic seed-coating agent. In addition, the toxicity of the novel agent was also lower than that of traditional ones. Therefore, the application of the novel agent for cucumber is an appropriate option for controlling pests and replacing high toxicity ones.**

Keywords seed-coating agent, natural polysaccharide, germination percentage, cucumber yield

Introduction

Cucumber is one of the main thermophilic vegetables, accounting for more than 15% of the total vegetable planting area in China. Now the biggest problem is chilling injury and pest damage in seedling stage, yet seed coating is the main method and key technology to control of pests and diseases (Xiong et al., 2004). To prevent considerable economic losses, seed-coating technology as a pesticide against crop pests and diseases has been widely used for crop protection. Results indicate that it can prevent the seeds from pests in the soil and plant and improve the germination potential, germination percentage, and crop yield (Ahmed et al., 2001). However, most of the seed-coating agents currently used are not the best alternatives for the environment due to their accumulative toxicity in the soil (Xie and Xu, 2008; Khalid et al., 2010).

The main objective of our study was to prepare an environmentally friendly cucumber seed-coating agent as an alternative to traditional toxic ones. The effect of the novel seed-coating agent (NS) on antifeedant rate, germinability, germination percentage, and toxicity was investigated. With the application of NS, cucumber seeds had better quality and properties that resulted in an increased yield. Results indicated that NS also could enhance the pest-resistant ability of plants. The foremost difference between NS and traditional ones was that it controlled pests not by killing pests but by both repelling pests and enhancing the immunity of seeds. Therefore, NS could bring obvious economic and environmental benefits.

Materials and methods

Experimental materials

Experimental materials used consisted of natural polysaccharide (prepared in laboratory), polyethylene glycol, 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.), traditional 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

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cucumber seeds (Jingyou 2, Wuhan Seed Co., Ltd., Tianhong, China).

Constant temperature and humidity incubator (Model No. WS-01, Hubei Huang Shi Hengfeng Medical Instrument 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.) were used along with the major instruments and glassware of 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 of 90 cm in diameter (Shanghai Yuejin Medical Treatment Instrument Plant).

Methods

Preparation of NS

The natural polysaccharide was prepared in 1% acetic acid to different concentrations and stirred at room temperature for approximately 3 to 5 h. The components have a molecular weight of 30 to 1400 kDa and deacetylation degree of 80% to 90%. The optimal formulation of the novel seed-coating agent was determined through orthogonal test. NS was prepared with the following components (%): natural polysaccharide 52, fertilizer 14, microelement 14, sodium hydroxide 6, ethylene glycol 3, polyethylene glycol 2, borax 1, film former 0.5, penetrating agent 1, violet pigment 0.5, and deionized water 6. Aqueous solutions of fertilizer, microelement, sodium hydroxide, ethylene glycol, borax, film former, penetrating agent, and violet pigment were prepared respectively at a concentration of 1 wt%. After being mixed in every component completely at room temperature, the purple suspension was gained as the working liquid of NS.

Laboratory method for antifeeding test

According to the guideline for laboratory bioassay of pesticides, the antifeeding effect of NS was studied in no choice bioassays with the artificial mixed feeding method and leaf discs method. The artificial mixed feeding method was as follows: 100 g artificial feed was individually dipped in different concentrations of NS solutions and then put into each hole with diameter of 1.5 cm and depth of 1.5 cm, where the fourth instar larvae were fed, with three replicates for each treatment. The leaf discs method was as follows: fresh cucumber leaf discs of 4 cm diameter were punched using a cork borer and individually dipped in different concentrations of NS solutions, with one fourth instar larva in each Petri dish. Artificial feed and leaf discs treated with water (CK) were used as controls, and each treatment was replicated three times. The antifeeding effect was determined at 48 h after breeding in the incubator at temperature of 25±1ºC and 75% to 85% relative humidity under a day length of 16L∶8D using the artificial mixed feed weight and a leaf area meter to measure the consumption. The test was valid only at less than 5% mortality of larvae. The non-selective antifeedant rate (AR) was calculated by the following formula:

$$AR(\%) = \frac{A-B}{A} \times 100\%,$$

where A and B are the consumption of the control group and the treatment group, respectively.

Laboratory method for germination abilities test

According to the rules for seed testing of International Seed Testing Association, 90 seeds from each group were arranged on two layers of wet filter paper in Petri dishes filled with wet sand. Each Petri dish contained 30 seeds and each treatment was replicated three times. All Petri dishes were incubated in the constant temperature and humidity incubator at 28±1ºC and air relative humidity of 85%. Germinability (GE) and germination percentage (GP) of cucumber seeds were investigated on the third and seventh days, respectively. The calculation formulae were as follows:

$$GE = \frac{C}{E} \times 100\%,$$

$$GP = \frac{D}{E} \times 100\%,$$

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 toxicological test

According to the toxicological test methods of pesticides for registration (The Chinese State Standard GB15670-1995), the toxic effect of NS and CA was studied with the LD_{50} method. The toxic materials may enter the body mainly via ingestion (gastrointestinal tract) and dermal contact. In the experiment, rats were fasted overnight before treatment, 14 days after treatment, 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 above toxicological test, we determined the differences in toxicity and safety, etc., between NS 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. The cucumber seeds were coated with each of the seed-coating agents in the proportion of 1∶50 (w/w), air-dried at room temperature for about 30 min, and then sown into the field. Each plot was sown with either coated seeds or uncoated seeds as control, and field management was the same for all experimental plots. A random sample of 100 seedlings was selected to determine the germination percentage (GP). Total yield of each plot was determined by

Table 1 Effects of different treatments on the consumption of artificial mixed feed and cucumber leaf area

Treatment	Mean artificial mixed feed consumption (g)	*AR* (%)	Mean leaf area consumption (mm^2)	*AR* (%)
1% NS	1.13±0.05b	50.44	956.4±65.43a	29.52
2% NS	0.91±0.05c	60.09	815.8±47.58e	39.87
3% NS	0.69±0.04e	69.74	611.5±46.52b	54.93
4% NS	0.42±0.03a	81.58	235.3±13.63c	82.66
Water	2.28±0.15b	—	1356.8±76.52b	—

Values are expressed as mean±standard error. Means with different letters are significantly differences at $P < 0.05$.

weighing 10 cucumbers selected random and finally the yield of per hectare was investigated.

Results

Results of antifeeding test

In all NS treatments against *Agrotis ypsilon*, the artificial mixed feed weight and mean leaf area consumption declined with the increase in the concentrations (Table 1) and were significantly less than those of the control group. Table 1 shows an excellent antifeedant effect based on the analysis of non-selective antifeedant rate of NS. The artificial feed without NS was nearly eaten up, while the artificial feed with NS was rarely touched.

Results of germination abilities test

Results related to germinability (GE) and germination percentage (GP) showed that the NS treatments resulted in a significant increase in GE and GP compared to the traditional seed-coating agent (CA) and the blank control group (CK) (Fig. 1). The formulation with the highest concentration of NS (4%) was the best treatment. However, no significant difference was observed among the treatments of 3% and 4% NS.

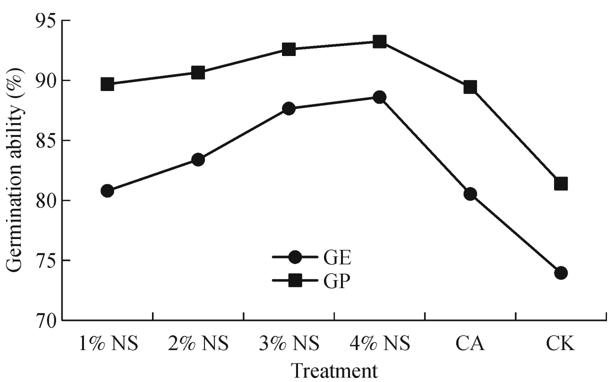

Figure 1 Effects of different treatments on germinability (GE) and germination percentage (GP).

Results of toxicological test

Results of toxicological test showed a significant difference in the LD_{50} between the two seed-coating agents (Table 2). In the acute oral toxicity test, rats infected with a high dosage of CA would exhibit some toxic symptoms after 3 to 6 min, including systemic muscle spasm, salivation, and even convulsions, and sticky nasal and ocular secretions in the 12 h before death. Rats infected with low dosages still showed slight muscle spasm. However, rats infected with NS did not exhibit the toxic symptoms mentioned above. In the acute skin toxicity test, rats were observed consecutively for 2 weeks after they were infected with the toxicant according to the conventional method. There was no significant difference between the median lethal dose (LD_{50}) to the male and that to the female rats for NS, and the acute toxicity of NS in this study was much lower than that of the traditional agent.

Results of field trial

Results of field trial showed that NS noticeably improved the main performance indices such as the germination percentage and the average yield per ha (Table 3). Table 3 shows the increase of 12.9% over CK and 9.3% over the CA-coated group in 2008. In 2009, the yield of NS-coated group was 13.7% higher than that of CK and 8.5% higher than that of CA-coated group. Furthermore, the cost of NS was 16.7% less than that of CA. These results confirmed that the novel seed-coating agent not only can significantly improve seed germination but also enhance cucumber yield.

Discussion

Mechanism of antifeedant effect

Natural polysaccharide has been applied to various fields, including agriculture, and has been shown to affect many plant responses (Nungruthai et al., 2010). Our research indicated that the NS acted as a seed-coating agent to control *Agrotis ypsilon* effectively. Natural polysaccharide stimulates plants to produce systematic antibodies, which can produce repellant effects to deter insect pests. In addition, NS with natural antifungal property can induce systemic resistance and produce significant action repellent to crop pests (Ma and He, 2001; Shibuya and Minami, 2001). The restraint of feeding behavior might be in that the information input sensor is interrupted. The odour from natural polysaccharide plays a barrier function in feeding behavior or directly acts on the

Table 2 Effects of different treatments on median lethal dose

Treatment	Gender	Acute oral toxicity LD50 (mg/kg)	Acute skin toxicity LD50 (mg/kg)	Toxicity classification
1% NS	male	895	2987	low-toxic
	female	786	2855	low-toxic
2% NS	male	879	2857	low-toxic
	female	783	2784	low-toxic
3% NS	male	865	2786	low-toxic
	female	781	2775	low-toxic
4% NS	male	859	2758	low-toxic
	female	781	2726	low-toxic
CA	male	209	778	moderate-toxic
	female	189	664	moderate-toxic

Acute oral toxicity grading scale: low-toxic: $LD_{50} > 500$ mg/kg; moderate-toxic: $50 < LD_{50} < 500$ mg/kg. Acute skin toxicity grading scale: low-toxic: $LD_{50} > 2000$ mg/kg; moderate-toxic: $200 < LD_{50} < 2000$ mg/kg (Chinese acute toxicity classification standard).

Table 3 Effects of different treatments on germination percentage and yield

Treatment	Germination percentage (%)		Yield (kg/hm^2)		Cost (US$/kg)
	2008	2009	2008	2009	
1% NS	91.2b	91.4b	215600c	203100b	1.5
2% NS	91.4a	89.2a	205400a	199600b	1.5
3% NS	92.6c	91.5b	223600b	215600b	1.5
4% NS	93.6b	92.2c	221400a	213600a	1.5
CA	84.5a	86.6a	204600b	198700b	1.8
CK	79.4b	78.8b	198000b	189600b	—

Values with different letters are significantly differences at $P < 0.05$ according to the Duncan's multiple range test.

nervous system of animals, which causes the unusual discharge of the nervous system and prevents animals from getting correct information of taste (Dayani et al., 2000; Takashi et al., 2004).

Mechanism of the yield increase effect

Natural polysaccharide has excellent film-forming property, making it easy to form a semi-permeable film on the seed surface that can maintain the seed moisture and absorb the soil moisture; thus, it can promote seed germination. In contrast, it can cut off excessive soil moisture to prevent the seed from rotting. Furthermore, 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 to restrain the seed respiration and thus to make the internal nutrient consumption of seeds fall to the lowest possible level (Furbank et al., 2004). This kind of semi-permeable film is believed to be able to maintain the seed moisture and absorb the soil moisture; thus, it can promote seed germination (William and Ronald, 2002). Natural polysaccharide also can increase soluble sugar content and enhance the activity of protease conversion to protein and increasing free amino acid content, which has obvious inhibiting effect for many plant pathogenic fungi. Natural polysaccharide as a novel plant disease inhibitor can induce and improve the disease resistance of plants and thus has an effect repellent to the pests in the soil (Chen and Xu, 2005). Natural polysaccharide possesses the natural antifungal role, which increases the permeability of the outer membrane and inner membrane and ultimately disrupts bacterial cell membranes, with the release of cellular contents (Nielsen et al., 1994; Liu et al., 2004).

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