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Full Length Research Paper

Effects of an environmentally friendly seed coating agent on combating head smut of corn caused by *Sphacelotheca reiliana* and corn growth

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Head smut of corn is caused by the fungus *Sphacelotheca reiliana* and occurs in the North -east of China and in regions of similar climate. Yield losses due to the disease are variable and directly depend on the severity of the disease. The objective of this study was to produce a coating technology to protect corn from *S. reiliana* and to avoid environmental pollution. The effects of seed coats on inhibitory index, germination percentage, seedling growth and crop yield of corn plant were investigated in the laboratory and field trials. Compared with the conventional toxic seed coating agent, the crop yield of seeds coated with the novel seed coating agent was increased by 11.6 - 14.6%. The fungal inhibition test of the novel seed coating agent showed that it had better fungal inhibitory effect. Our findings indicated that the application of the novel seed coat had a remarkable effect on the resistance to head smut of corn and yield enhancement.

Key words: Head smut of corn, seed coating agent, inhibitory index, germination percentage**,** corn yield.

INTRODUCTION

In recent decades, corn has become an attractive and promising commodity due to its multifunctional uses as an animal feed, industrial material and a human food. Head smut of corn, caused by the fungus *Sphacelotheca reiliana* is a serious disease of corn in temperate regions throughout the world, including the northeast of China. It has caused significant yield losses in areas such as Heilongjiang, Jilin and Liaoning. The disease currently is managed by the use of a seed coating agent (AMULET). Seed treatments are used to incorporate pesticides onto the seed coat and to decrease the disease susceptibility of the seed during its germination in the soil (Freeborn et al., 2001). Studies have shown that a seed coating is effective in preventing and controlling mold-induced diseases and the pests causing them, promoting seedling growth and increasing yields (Wang, 2001; Richard, 2005; Qiu et al., 2005; Russ and David, 2005; Song et al., 2005). Seed coating technology has developed rapidly during the past two decades and provides an economical approach to seed enhancement, especially for larger seeded agronomic and horticultural crops. An advantage of seed coating is that the seed enhancement material (fungicide, insecticide and micronutrient) is placed directly on the seed without obscuring the seed shape (Ehsanfar and Mdarre, 2005). Although scientists have developed a number of coating compounds that protect corn from smut fungus, all research on seed coatings in the past have been limited to toxic fungicides such as carbofuran, thiram and triadimenol. Among those poisonous constituents, a dose of 7 mg carbofuran is lethal and the period of residual toxicity of it in the soil is up to 50 years (Zhu et al., 2003; Wang et al., 2005; Xu and Zhu, 2005; Da, 2006). Such substances bring with them threats varying from acute to chronic health effects on humans and animals, making the development of eco-agricultural practices impossibility. The dreaded threat of current conventional toxic seed coating agent demands a new generation of corn seed coatings that can also offer guarantees to corn yield and environmental protection.

Among other polymers, chitosan has received significant attention as an antimicrobial film-forming agent for food preservation purposes due to its biodegradability, bio-compatibility, cytotoxicity and antimicrobial activity (Dutta

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et al., 2009). Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin, a major component of the shells of crustacea such as crab, shrimp and crawfish (Muzzarelli, 1977; Knorr, 1984). In this regard, we have used modified chitosan successfully in the preparation of a novel corn seed coating agent (NCSCA) instead of the conventional toxic fungicide. Compared with a conventional seed coating (AMULET), the effect of NCSCA on the resistance to head smut, seed germination and seedling growth, as well as corn yield were studied.

MATERIAL AND METHODS

Materials

The constant temperature and humidity incubator (WS-01#, CTHI) was obtained from Hengfeng Medical Device Factory, China. The high pressure steam sterilizing vessel (YXQ-SG46-48SA#, HPSSV) was purchased from Shanghai BoXun, Inc., China. The biomicroscope (BX41- 12HO2#) was provided by OLYMPUS Co., Japan. The culture dish (90 cm) was received from YueJin Medical Device Factory, China

Modified chitosan, ethylene glycol, polyethylene glycol 1000, sodium hydroxide, filmerformer, blue pigment, zinc, manganese, copper, molybdenum, potassium and other trace elements were of analytical grade. Potato dextrose agar (PDA), corn seeds (Y8#) and smut fungus samples were received from Jilin Academy of Agricultural Sciences, China. AMULET, 20% thiram/carbofuran seed coating agent for corn, was obtained from China Seed Group Co.

Experimental plots

All field tests were conducted at the Gongzhuling Corn Experimental Base (43.4°N, 124.49°E, naturally *S. reiliana*-infected) of the Jilin Agriculture and Science Academy, P.R. China and Heilongjiang Corn Experimental Base(46.59°N, 128.01°E, naturally *S. reiliana*-infected), P.R. China.

Preparation of the novel seed coating agent

The modified chitosan has a molecular weight of 200 kD and deacetylation degree of 85%. Chitosan solution was prepared at 1% (w/w) in 1% (w/w) aqueous HAc. It takes about 3 - 5 h to dissolve modified chitosan completely under stirring at 25°C. Aqueous solutions of polyethylene glycol, sodium hydroxide and trace elements were prepared at a certain concentration. All the reagents were mixed into the vessel according to a certain proportions and order. After mixing in every component completely at room temperature, the working liquid was a purple suspension.

The optimal coating formulation was determined through orthogonal test. The novel coating (NCSCA) is perpared with the following main compoments (w/w): 1% modified chitosan (85%), trace elements (5%), ethylene glycol (3%), polyethylene glycol (3%), filmerformer (0.5%), purple pigment (3.5%). The pH of the mixture was adjusted to 6.0 with 2% (w/w) NaOH.

Assay of antifungal activity of seed coating agents

This assay was done according to the method of D. Jasso de Rodríguez and co-workers with some modifications (Rodríguez et al., 2005). Different concentrations of seed coating agents were, respectively, added to sterilized potato dextrose agar (PDA). Then, the test petri dishes were incubated at 27°C after transferring the mycelium of fungi. When the mycelium of fungi reached the edges of the control petri dish (without added seed coating agent), the antifungal index was calculated as follows:

$$\text{Inhibitory index } (\%) = (A - B)/A \times 100\% \quad [1]$$

Where A is the diameter of the growth zone in the control petri dish and B is the diameter of the growth zone in the test petri dishes. Each experiment was performed three times and the data were averaged.

Laboratory method for the germination test

Corn seeds for examination were coated with NCSCA and AMULET by hand in the proportion of 50:1 (wt. %), respectively, and then dried by airing for 20 min to prepare for use. Uncoated corn seeds (CK) were prepared as the blank control group. According to the rules for seed testing of the International Seed Testing Association (ISTA, 2006), 50 seeds taken from each group were arranged on two layers of wet filter paper in each culture dish (90 mm) filled with paddy soil with 3 replications and incubated in the constant temperature and humidity incubator at 25 ± 1°C and air relative humidity of 85%. Seeds were considered germinated when radicles emerged. Seeds without protruded radicle tip were considered as nongerminated. The germinability and germination percentage (GP) of corn seeds were investigated on the third day and seventh day, respectively. The seedlings quality was determined on the seventh day. The calculation formulas are as follows:

$$\text{Three day germination } (\%) = (GS3/TS) \times 100\% \quad [2]$$

$$\text{Germination Percentage } (\%) = (GS7/TS) \times 100\% \quad [3]$$

Where GS3 is the number of germinated seeds on the third day, GS7 is the number of germinated seeds on the seventh day and TS is the number of total seeds investigated.

Field test

A preliminary field test to determine seed germination and corn yield was conducted in Gongzhuling on 15 May, 2006. All test seeds were coated by hand at a ratio of 1:50 (w/w). After spreading and airing for 30 mins at room temperature. The experiments were designed as a randomized block design with each treatment consisting of a plot of 20 m^2 (2 × 10 m) had 20 rows 50 cm apart with a plant spacing of 20 cm. 200 corn seeds were planted for each plot. The treated and uncoated seeds were, respectively, sown in randomly arranged plots with 3 replications. Each Field management was the same for all experimental plots. The germination percentage, plant height and inhibitory index were investigated. Total yield for each plot was determined by weighing the seeds of established plants of the middle four rows after threshing.

Three separate, replicated experiments were conducted in Gongzhuling in 2007 and in Heilongjiang in 2006 and 2007, respectively. The experimental methods were the same as described above.

Statiscal analysis

All the quantitative estimations were analyzed and the values were expressed as mean ± standard error. The data were statistically

Table 1. Inhibition effect of seed coating agent against *Sphacelotheca reiliana.*

Agent	Concentration (µgml $^{-1}$)	Colony diameter (mm)	Inhibitory index %
AMULET	0	77.0	-
	500	42.5	44.8
	1000	32.5	57.79
	1500	19.7	74.42
	2000	10.2	86.75
NCSCA	0	77.6	-
	500	20.6	73.45
	1000	16.8	78.35
	1500	13.4	82.73
	2000	6.7	91.37

Table 2. Effects of seed coating agents on seed germination and seedling growth.

Treatment	Germinability (%)	Germination Percentage (%)	Shoot height (cm)	Root length (cm)	Sprout length (g)	Dry weight of shoot (mg)	Dry weight of root (mg)
NCSCA	85.8a ± 0.8988	98.7a ± 1.5394	12.18a ± 0.25	13.68a ± 0.30	4.29a ± 0.15	41.25a ± 0.02	22.23a ± 0.02
AMULET	73.0b ± 0.9643	92.1a ± 2.1166	11.92a ± 0.25	13.53a ± 0.12	4.08a ± 0.17	40.33a ± 0.02	21.78a ± 0.02
CK	64.1c ± 1.1135	82.8b ± 2.6665	10.54b ± 0.25	10.57b ± 0.12	3.41b ± 0.17	36.85b ± 0.02	18.48b ± 0.02

Values are expressed as mean ± standard error (n=20), Means designated with different letters are significance different at $p < 0.05$.

analyzed by Duncan's multiple range tests as available on the SPSS 12.0 statistical pachage. Significant effects of treatments were determined by the magnitude of P value (P = 0.05).

RESULTS

Antifungal activities of seed coating agents

The antifungal activities of seed coating agents are shown in Table 1. As shown in Table 1, AMULET has antifungal activity against *S. reiliana* and the inhibitory index is 44.8, 57.79, 74.42 and 86.75% at 500, 1000, 1500 and 2000 µgml^{-1}, respectively. Compared with the activity of AMULET, NCSCA has better antifungal activity and the inhibitory index is 73.45, 78.35, 82.73 and 91.37% at 500, 1000, 1500 and 2000 µgml^{-1}, respectively.

Effect of seed coating agents on seed germination and seedling vigor

Germination was observed in all the seeds coated with different seed coats. Among the different treatments of corn seeds, NCSCA recorded maximum germination. NCSCA treatment increased the vegetative growth parameters of corn. NCSCA treatment significantly increased the shoot height, root length, sprout length and dry weight of seedling (Table 2).

Effect of seed coating agents on head smut disease incidence under field condition

The results indicated that NCSCA improved the main performance indexes such as the germination percentage, plant height and crop yield (Table 3 and Table 4). It could also protect corn plant against *S. reiliana* efficiently. The statistical analysis showed that there was significant difference among the three treatments in corn yield. NCSCA showed increases of 14.2 and 20.5% to the AMULET-coated group and CK, respectively.

DISCUSSION

The fungistatic mechanism of NCSCA

The above results indicated that NCSCA had a positive effect on the resistance to *S. reiliana*. This is due to the use of chitosan. A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier (Rabea et al., 2003). One of the reasons for the antimicrobial character of chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi et al., 1999). Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of

Table 3. Effect of seed coating agents on corn growth.

Time and region	Treatment	Main Performance Indexes		
		Germination Percentage (%)	Plant Height (cm)	Inhibitory Index (%)
2006 in Gongzhuling	NCSCA	95.2a ± 0.63	212.3a ± 13.1	92.a8 ± 0.54
	AMULET	91.0b ± 0.54	198.5b ± 11.8	87.3b ± 0.50
	CK	80.3c ± 0.78	195.1b ± 12.7	52.4c ± 0.06
2007 in Gongzhuling	NCSCA	96.6a ± 0.0.58	208.5a ± 12.6	92.3a ± 0.63
	AMULET	92.1b ± 0.64	203.0b ± 14.2	87.6b ± 0.85
	CK	81.5c ± 0.85	198.8c ± 10.3	59.8c ± 0.03
2006 in Heilongjiang	NCSCA	91.3a ± 0.57	204.3a ± 11.5	92.9a ± 0.57
	AMULET	90.5b ± 0.69	197.4b ± 12.1	90.1a ± 0.69
	CK	79.9c ± 0.75	198.7b ± 13.1	58.7b ± 0.05
2007 in Heilongjiang	NCSCA	92.6a ± 0.42	198.6a ± 12.0	91.5a ± 0.64
	AMULET	89.4b ± 0.51	193.5b ± 12.4	88.3b ± 0.58
	CK	78.8c ± 0.65	192.4b ± 11.4	59.0c ± 0.03

Seeds were sown on May 25th and the germination percentage was investigated on June 25tth, the plant height was investigated five days before harvest. The inhibitory index was determined by the percentage of plant not infected by *S. reiliana.* Values are expressed as mean ± standard error (n = 20), Means designated with different letters are significance different at $p < 0.05$.

Table 4. Effect of seed coating agents on corn yield.

Time and region	Treatment	Mean yield per plot (kg)
2006 in Gongzhuling	NCSCA	28.339a ± 0.1988
	AMULET	25.234b ± 0.6117
	CK	23.945c ± 0.084
2007 in Gongzhuling	NCSCA	28.328a ± 0.1712
	AMULET	24.929b ± 0.0454
	CK	23.209c ± 0.3535
2006 in Heilongjiang	NCSCA	27.951a ± 0.0773
	AMULET	24.534b ± 0.2881
	CK	23.018b ± 0.0746
2007 in Heilongjiang	NCSCA	28.029a ± 0.2673
	AMULET	23.932b ± 0.0909
	CK	23.322b ± 0.2129

Values are expressed as mean ± standard error n=3 , Means designated with different letters are significance different at $p < 0.05$.

toxins and microbial growth (Cuero et al., 1991). It also activates several defence processes in the host tissue (Ghaouth et al., 1992), acts as a water binding agent and inhibits various enzymes. Binding of chitosan with DNA and inhibition of mRNA synthesis occurs through chitosan penetration toward the nuclei of the microorganisms and interference with the synthesis of mRNA and proteins (Sudarshan et al., 1992). It has been proposed that when chitosan is liberated from the cell wall of fungal pathogens by plant host hydrolytic enzymes, it then penetrates to the nuclei of fungi and interferes with RNA and protein synthesis (Hadwiger et al., 1985).

Effect of NCSCA on yield attributes

Firstly, Seed coating is an on-seed delivery mechanism that provides all the advantages and more of a starter fertilizer. The ingredients wrapped around the seed include plant nutrients, a plant growth regulating agent, bio-fungicides and an energy supply. The field experiment results showed that seeds coated with NCSCA had better resistance to *S. reiliana* and higher germination percentage than the control. The modified chitosan contained in NCSCA enhanced the resistance to *S. reiliana* increased seed germination and improved seedling growth throughout the corn growing season.

Secondly, Modified chitosan is a copolymer of glucosamine and N-acetyglucosamine units linked by 1-4 glucosidic bonds. It has excellent film-forming property, making it easy to form a compact protective film on the seed surface which can delay the release of fertilizer elements, reduce nutrient losses and significantly improve fertilizer efficiency. Furthermore, the film with good permeability not only guarantees enough water and oxygen for corn seeds, but also lays the foundation for sustained release of active ingredients (Li and Wu, 2004; Robert et al., 2004).

Another factor that contributes to the corn yield increase may be the effect of the trace elements contained in NCSCA. The trace fertilizer can increase the enzymatic activity of the corn seed, which is helpful to the transformation of protein into amino acids and starch into simple sugars or fat to provide abundant nutrient sources for germs to grow.

CONCLUSION

Our laboratory and field experiments describe a novel corn seed coating agent (NCSCA). Under *S. reiliana* stress, NCSCA maintained a high resistance to *S. reiliana* and high germination percentage, which both provide a guarantee to enhance corn yield. Furthermore, NCSCA is made of a natural nontoxic biopolymer, trace elements and fertilizer. It has achieved the goals of resisting head smut, enhancing corn yield and avoiding environmental pollution. It will produce obvious economic and environmental benefits when this novel seed coating agent is used to replace the conventional and toxic corn seed coating agent in agriculture.

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