STUDY AND PREPARATION OF AN ENVIRONMENTALLY FRIENDLY CORN SEED COATING AGENT

Defang Zeng, Xiang Mei, Juanjuan Wu*

School of Resource and Environmental Engineering, Wuhan University of Technology, Wuhan 430070, P.R. China

Received: October 10, 2009
Accepted: April 25, 2010

Abstract: Head smut of corn is caused by the fungus *Sphacelotheca reiliana* and occurs in northeast China and in regions of a 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 head smut and to avoid environmental pollution. Based on its excellent properties of high efficiency, nonpollution and nontoxicity, a novel seed coating agent was prepared with modified chitosan as the main material and trace elements and fertilizer as the auxiliary material. Compared with the conventional toxic seed coating agent, the novel seed coating agent protected the seeds and provided excellent control of head smut and increased yield by 11.6 to 14.6%, while the cost of seed coating agent decreased by 32.4%. Our findings indicate that the application of chitosan in seed coating technology had a remarkable effect on the resistance to head smut of corn and yield enhancement.

Key words: head smut of corn, seed coating agent, corn yield, germination percentage, inhibitory rate

INTRODUCTION

In recent decades, corn had become an attractive and promising commodity due to its multifunctional uses as an animal feed, industrial material, and a human crop. 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 caused significant yield losses in the areas such as Heilongjiang, Jilin and Liaoning. The disease currently is managed by the use of a seed coating agent. 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 mould-induced diseases and 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 developed rapidly during the past two decades and provides an economical approach to seed enhancement, especially for larger seeded agronomic and horticultural crops. The 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 developed a number of coating compounds that protect corn from smut fungus, all research on seed coatings in the past was limited to toxic fungicides such as carbofuran, thiram, and triadimenol. Among those poisonous constituents, a dose of only 7 mg carbofuran is lethal and the period of residual toxicity of carbofuran in the soil is up to fifty years (Zhu *et al*. 2003; Wang *et al*. 2005; Xu and Zhu 2005; Da 2006). Such substances bring threat varying from acute to chronic health effects on human, and animals and make the development of eco-agricultural practices impossible. 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. In this regard, we successfully used a modified preparation of a novel corn seed coating agent (short for MCSCA) instead of conventional toxic fungicide. Compared with a conventional seed coating (AMULET), the effect of MCSCA on the resistance to head smut, seed germination, and corn yield, as well as its toxicity was studied.

MATERIALS AND METHODS

Materials and chemicals

Modified chitosan, ethylene glycol, polyethylene glycol 1 000, sodium hydroxide, potato dextrose agar, filmer-former and trace elements were of analytical grade. AMULET, 20% thiram/carbofuran seed coating agent for corn, was obtained from China Seed Group Co. Corn seeds and smut fungus samples were received from China Jilin Seed Co. Sprague-Dawley rats (20 males, 20 females; 187±12 g) were supplied by the animal center of TongJi Hospital.

Experimental plots

All field tests were conducted at the Gongzhuling Corn Experimental Base (naturally *Sphacelotheca reiliana* infected) of the Jilin Agriculture and Science Academy,

*Corresponding address:
yangyu.86@hotmail.com

P.R. China, and Heilongjiang Corn Experimental Base (naturally *S. reiliana* infected), P.R. China.

Preparation of MCSCA

A modified chitosan was prepared as 1.0 (wt %): 1.0 (wt %) aqueous HAc. It takes about 3–5 h to dissolve modified chitosan completely under stirring at 25. The components have a molecular weight of from 20 to 1 500 kD, deacetylation degree of from 75 to 90%.

The optimal coating formulation was determined through orthogonal test. The novel coating (MCSCA) is prepared with the following main components (wt %) : 1% modified chitosan (65), trace elements (25), ethylene glycol (3), polyethylene glycol (3), filmformer (0.5), purple pigment (3.5), pH of the mixture was adjusted to 6.0 with 2.0 wt. % NaOH. After mixing in every component completely at room temperature, the working liquid of MCCSA was a purple suspension.

Laboratory method for fungistatic effect

Potato dextrose agar was sterilised with a high-pressure steam, inoculated with *S. reiliana*, with MCSCA and AMULET added respectively, and cultivated in a constant temperature and humidity incubator for 72 h (Zhao and He 2005). The fungistatic effect was observed under the biomicroscope and the diameter of colony was measured twice. The average value was recorded. The experiments were repeated three times. The inhibitory rate was calculated by the following formula (Sun *et al.* 2004):

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

A denotes the presence of fungi in the control and B denotes the presence of fungi in the treated variant.

Laboratory method for the germination test

Corn seeds for examination were coated with MCSCA 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 (short for CK) were prepared as blank control group. 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 culture dish (Φ90 cm) filled with paddy soil with three replications, and incubated in the constant temperature and humidity incubator at 15±1°C and air relative humidity of 85%. The germinability and germination percentage (GP) of corn seeds were investigated on the third day and seventh day, respectively. The calculation formulas were as follows:

$$\text{Germinability}(\%) = \frac{GS3}{TS} \times 100\% \quad (2)$$

$$\text{Germinability}(\%) = \frac{GS7}{TS} \times 100\% \quad (3)$$

GS3 is the number of germinated seeds on the third day; GS7 is the number of germinated seeds on the seventh day; 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 in 2006. In this test, the method of seed treatment was the same as that in the laboratory experiment. The treated and uncoated seeds were, respectively, sown in randomly arranged plots with 3 replications. Each plot of 20 m^2 (2 m x 10 m) had 6 rows with a spacing of 60 cm. Field management was the same for all experimental plots. The corn yield was recorded at harvest.

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.

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 MCSCA and AMULET were studied with the Horn method. The $LD_{50,}$ the most widely used index of acute toxicity, was determined.

SD rats were acclimatized for 5 days before experimentation and then were randomly divided into 4 groups, 10 in each group, half male and half female. The animals were fasted overnight before treatment. There were two major exposure routes by which toxicity materials may enter the body: ingestion (gastrointestinal tract) and dermal contact (skin). (1) In acute oral toxicity test rats were fed by garage through the mouth with MCSCA and AMULET, respectively. 14 days later, the toxic symptoms and LD_{50} were recorded. (2) In acute skin toxicity test the rats had their hair removed before the trial. 24 hours later, MCSCA and AMULET were smeared on the shed locations on the rats, respectively. 14 days later, the toxic symptoms and LD_{50} were recorded.

RESULTS

Results of resistance to *S. reiliana* and seed germination in the laboratory

Table 1 shows that CK had a high risk for *S. reiliana* infection, and the inhibitory rate of MSCSA-treated seeds was up to 97.77%, higher than that of the seeds coated with AMULET (87.87%). It indicated that both seed coating agents can obviously protect seeds from *S. reiliana*. The effect of these two seed coating agents on the resistance to *S. reiliana* was different. However, MCSCA was the better one.

Furthermore, germination energy and germination percentage of corn seeds were determined by inhibitory effects of the seed coating agents. The average level of germinability of seeds coated with MCSCA was 85.77%, with a range from 84.3% to 87.4%, about 17.5% higher than that of seeds coated with AMULET and almost 33.9% higher than that of CK. Similar results were recorded with respect to germination percentage. Seeds coated with MCSCA showed 7.2% and 19.2% increase in germination percent-

Table 1. Results of comparative experiments among different treated seeds in the laboratory

Test, replication	Germinability [%]	GP [%]	Inhibitory rate [%]
MCSCA treated			
1	84.3	98.0	98.3
2	85.6	99.7	98.2
3	87.4	98.4	96.8
Mean	85.8	98.7	97.8
SE	0.8988	1.5394	1.4525
AMULET treated			
1	73.3	91.0	87.2
2	74.5	93.4	88.0
3	71.2	91.8	88.4
Mean	73.0	92.1	87.9
SE	0.9643	2.1166	1.0583
CK			
1	64.8	84.5	–
2	63.8	81.5	–
3	63.6	82.4	–
Mean	64.1	82.8	–
SE	1.1135	2.6665	

Explanation – values within a column followed by different letters are significance different (p = 0.01) according to the Duncan's multiple range test; SE – Standard error; CK – Blank control; MCSCA – novel seed coating agent; AMULET – conventional seed coating agent

Table 2. Yield results of the field test

Treatment	Yield per plot [kg]												Mean	SE
	2006 in Gongzhuling			2007 in Gongzhuling			2006 in Heilongjiang			2007 in Heilongjiang				
	1	2	3	1	2	3	1	2	3	1	2	3		
MCSCA	28.356	28.674	27.986	28.486	27.986	28.512	28.104	27.854	27.896	27.589	27.986	28.512	28.162	0.0387
AMULET	25.937	25.749	24.015	24.924	24.853	25.010	25.010	24.578	24.015	23.825	23.859	24.113	24.657	0.0645
CK	23.826	23.901	24.107	23.012	23.896	22.720	23.154	22.897	23.003	23.178	23.741	23.047	23.373	0.0258

Explanation: values within a column followed by different letters are significantly different (p = 0.01) according to the Duncan's multiple range test; MCSCA – novel seed coating agent; CK – Blank control

Table 3. Acute toxicities of AMULET in rats

Species	Route	LD_{50} [mg/kg]	Toxicity rating
Rat (male)	Oral	209	moderately toxic
Rat (female)	Oral	183	moderately toxic
Rat (male)	Dermal	756	moderately toxic
Rat (female)	Dermal	631	moderately toxic

Acute oral toxicity grading scale: (1) Slightly toxic: $LD_{50} > 500$ mg/kg; (2) Moderately toxic: $50 < LD_{50} < 500$ mg/kg; (3) Highly toxic: $LD_{50} < 50$ mg/kg
Acute dermal toxicity grading scale: (1) Slightly toxic: $LD_{50} > 2000$ mg/kg; (2) Moderately toxic: $200 < LD_{50} < 2000$ mg/kg; (3) Highly toxic: $LD_{50} < 200$ mg/kg

Table 4. Acute toxicities of MCSCA in rats

Species	Route	LD_{50} [mg/kg]	Toxicity rating
Rat (male)	oral	852	slightly toxic
Rat (female)	oral	763	slightly toxic
Rat (male)	dermal	2 543	slightly toxic
Rat (female)	dermal	2 469	slightly toxic

Acute oral toxicity grading scale: (1) Slightly toxic: $LD_{50} > 500$ mg/kg; (2) Moderately toxic: $50 < LD_{50} < 500$ mg/kg; (3) Highly toxic: $LD_{50} < 50$ mg/kg
Acute dermal toxicity grading scale: (1) Slightly toxic: $LD_{50} > 2000$ mg/kg; (2) Moderately toxic: $200 < LD_{50} < 2000$ mg/kg; (3) Highly toxic: $LD_{50} < 200$ mg/kg

age compared with AMULET-treated seeds and CK, respectively. These results showed that seed coating agents could significantly improve the germination percentage of seeds and the efficiency of MCSCA was obviously superior to AMULET in improving seed germination.

Results of the field test

The comparative results between MCSCA and AMULET at the Gongzhuling Corn Experimental Base of Jilin Agriculture and Science Academy are shown in table 2. It is concluded that MCSCA-coated group was the best compared with the other groups for corn output, which showed the increase of 18.4% over CK and 12.3% over the AMULET-coated group in 2006. In 2007, the output of MCSCA-coated group was 22.1% higher than that of CK and 13.6% higher than that of AMULET-coated group.

The field experiment results from the Heilongjiang Corn Experimental Base are shown in table 2. It is demonstrated that, in 2006, the yield of MCSCA-coated group was 21.4% higher than that of CK and 13.9% higher than that of AMULET-coated group. In 2007, the corn yield of MCSCA-coated group was 20.2% higher than that of CK and 17.1% higher than that of the AMULET-coated group, respectively.

These results confirmed that seed coating agents can, not only significantly improve seed germination but can also enhance corn yield. The statistical analysis showed that there was significant difference among the three treatments in corn yield. MCSCA showed the increase of 14.2% and 20.5% to the AMULET-coated group and CK, respectively. Furthermore, the cost of MCSCA (1.05 $/kg) was 32.4% less than that of AMULET (1.70 $/kg). So the ratio of performance to price for MCSCA is much higher than that of AMULET.

Results of toxicity test

AMULET turned out to be extraordinarily toxic to rats, producing muscle spasm, salivation, edema, and death. The acute oral LD_{50} and acute dermal LD_{50} in female rats were 183 mg/kg and 631 mg/kg, respectively. In laboratory rats, exposure to a low level (215 mg/kg) of AMULET caused a painful skin inflammation, while a high dose (2150 mg/kg) was found to be teratogenic and lead to developmental abnormalities. Concerning MCSCA and AMULET, we see that AMULET is about 3 times more toxic than MCSCA when measured in rats. Tables 3 and 4 show that toxicity of MCSCA is significantly lower than that of AMULET.

DISCUSSION

The fungistatic mechanism of MCSCA

The above results indicated that MCSCA had a positive effect on resistance to *S. reiliana*. That is mainly due to the use of chitosan. A number of studies on the antimicrobial characteristics of films made from chitosan were carried out earlier. Among other polymers, chitosan received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity (Dutta *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). The exact mechanism of the antimicrobial action of chitosan is still not well known, but different mechanisms were proposed (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 toxins and microbial growth (Cuero *et al.* 1991). It also activates several defense processes in the host tissue (El 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 was 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 MCSCA on yield attributes

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, plant growth regulating agent, bio-fungicides, and an energy supply. The field experiment results showed that seeds coated with MCSCA had better resistance to *S. reiliana* and higher germination percentage than the control. The modified chitosan contained in MCSCA enhanced the resistance to *S. reiliana*, increased seed germination, and improved seedling growth throughout the corn growing season.

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 foundation for sustained release of active ingredient (Li and Wu 2004; Robert *et al.* 2004).

Another reason contributes to the corn yield increase may be the effect of the trace elements and fertilizer contained in MCSCA. The trace fertilizer can increase the enzymatic activity of corn seed, which is helpful in the transformation of protein into amino acids and starch into simple sugar or fat to provide abundant nutrient sources for germ growing.

CONCLUSIONS

Our laboratory and field experiments described a novel corn seed coating agent (MCSCA). Under *S. reiliana* stress, MCSCA maintained a high resistance to *S. reiliana* and high germination percentage, which both provide

a guarantee to enhance the corn yield. Compared with AMULET, MCSCA-treated corn seeds showed a 14.2% increase in yield and a 32.4% decrease in cost. Furthermore, MCSCA 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 used to replace the conventional, and toxic, corn seed coating agents in agriculture.

ACKNOWLEDGEMENTS

The authors express their thanks to Wuhan University of Technology for financial support.

REFERENCES

Cuero R.G., Osuji G., Washington A. 1991. N-carboxymethyl chitosan inhibition of aflatoxin production: Role of zinc. Biotechnol. Lett. 13: 441–444.

Da Z.J. 2006. Development and application of the seed coating agent. Agric. Mark Weekly 25: 30–32.

Dutta P.K., Shipra T., Mehrotra G.K., Joydeep D. 2009. Perspectives for chitosan based antimicrobial films in food applications. Food Chem. 114: 1173–1182.

Ehsanfar S., Mdarre S. 2005. Crop protection by seed coating. Commun. Agric. Appl. Biol. Sci. 70: 225–229.

El Ghaouth A., Arul J., Asselin A., Benhamou N. 1992. Antifungal activity of Chitosan on post-harvest pathogens: Induction of morphological and cytological alternations in Rhizopus stolonifer. Mycol. Res. 96: 769–779.

Freeborn J.R., Holshouser D.L., Alley M.M., Powell N.L., Orcutt D.M. 2001. Soybean yield response to reproductive stage soil-applied nitrogen and foliar-applied boron. Agron. J. 93: 1200–1209.

Hadwiger L.A., Kendra D.F., Fristensky B.W., Wagoner W., Muzzarelli A., Jeuniaux C., Gooday G.W. 1985. Chitin in Nature and Technology. Plenum Publishers, New York: 210–216.

Knorr D. 1984. Use of chitinous polymers in food – a challenge for food research and development. Food Technol. 38: 85–97.

Lu C.Y., Wu W.J. 2004. Study on the effect of rape seed treated by coatings. Jiangsu Agric. Sci. 4: 21–23.

Muzzarelli R.A. 1977. Chitin. Oxford University Publishers, Oxford: 220–228.

Qiu J., Wang R.M., Yan J.Z. 2005. Seed film coating with uniconazole improves rape seedling growth in relation to physiological changes under water logging stress. Plant Growth Regul. 47: 75–81.

Rabea E.I., Badawy M.E.T., Stevens C.V., Smagghe G., Steurbaut W. 2003. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4: 1457–1465.

Richard M. 2005. Application of latex emulsion polymers in seed coating technology. Pestic. Formul. Appl. Syst. 23: 55–67.

Robert T.F., Rosemary W., Jairo A.P. 2004. Internal recycling of respiratory CO_2 in pods of chickpea (*Cicer arietinum* L.): The role of pod wall, seed coat, and embryo. J. Exp. Bot. 55: 1687–1696.

Russ W.G., David W.A. 2005. Influence of sowing date on emergence characteristics of maize seed coated with a temperature-activated polymer. Agron. J. 97: 1543–1550.

Shahidi F., Arachchi J.K.V., Jeon Y.J. 1999. Food application of chitin and chitosans. Trends Food Sci. Technol. 10: 37–51.

Song W.J., Hu J., Qiu J. 2005. Primary study on the development of special seed coating agents and their application in rice (*Oryza saliva* L.) cultivated by direct seeding. J. Zhejiang Univ. 31: 368–373.

Sudarshan N.R., Hoover D.G., Knorr D. 1992. Antibacterial action of chitosan. Food Biotechnol. 6: 257–272.

Sun X.H., Wang H.F., Liu Y.F. 2004. Synthesis of a new fungicide pyrimethanil. Org. Chem. 24: 506–510.

Wang C.C. 2001. Influence of seed coating formulations on maize production. Agric. Sci. Technol. 4: 22–24.

Wang Z.Q., Ning M.Y., He Y.Q. 2005. The development, promotion and application prospects of seed coating technology in China. Seed Ind. Rep. 4: 15–16.

Wu X.H., Zhang W.H., Liu P.F. 2003. Research and development trend of the seed coating agent in China. Plant Protect. Promot. Technol. 10: 36–38.

Xu Y.M., Zhu W.D. 2005. Pesticides Quiz. Chemical Industry Press, Beijing: 8–13.

Zhao B., He S.J. 2005. The Microbiological Experiments. Science Publishers, Beijing: 128–130.

Zhu Z.L., Gong R.Z., Han Z.H. 2003. The toxicity of pesticides on birds and the safety evaluation. Rural Ecol. Environ. 19: 53–57.

POLISH SUMMARY

BADANIE I PRZYGOTOWANIE PRZYJAZNEJ ŚRODOWISKU ZAPRAWY NASIENNEJ DLA KUKURYDZY

Grzyb *Sphacelotheca reiliana*, sprawca głowni pylącej kukurydzy, występuje w północno-wschodnich Chinach oraz na terenach o podobnym klimacie. Straty plonu są zmienne, choć bezpośrednio zależą od nasilenia choroby. Przedmiotem badań było opracowanie technologii zaprawiania nasion przeciwko głowni kukurydzy. Bazując na doskonałych właściwościach chitozanu, jego wysokiej efektywności, nie zanieczyszczaniu środowiska oraz braku toksyczności, przygotowano nową zaprawę ze zmodyfikowanym chitozanem jako składnikiem podstawowym oraz śladowymi pierwiastkami i nawozem, pełniącymi rolę pomocniczą. W porównaniu do konwencjonalnej, toksycznej zaprawy, nowa zapewniała doskonałe zwalczanie głowni kukurydzy i powodowała wzrost plonu od 11,6 do 14,6%, podczas gdy koszt zaprawy był mniejszy o 32,4%. Wykorzystanie chitozanu w technologii zaprawiania nasion podnosi odporność kukurydzy na głownię oraz wpływa na wzrost plonu.