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APPLICATION OF A NATURAL AMINOPOLYSACCHARIDE IN SEED FILM-COATING FOR PEST CONTROL AND COTTON GROWTH

ABSTRACT

Cotton suffers attacks of various pests that result in a decreased yield. Until recently, the chemical control of pests was achieved through seed film-coating treatment with toxic insecticides. The natural aminopolysaccharide extracts from crab is a promising candidate as a repellent to protect cotton from *Aphis gossypii* Glover and *Helicoverpa armigera* Hubner. Experiments were conducted to study the effect of aminopolysaccharide on pest control, seed germination, plant growth and lint yield. Results indicated that all aminopolysaccharide treatments of different content repelled pests efficiently and increased significantly seed germination, plant growth and cotton yield. The natural aminopolysaccharide extracts from shrimp is a promising candidate as a repellent to protect cotton from *Aphis gossypii* Glover and *Helicoverpa armigera* Hubner. The application of the potential aminopolysaccharide by seed coating is an appropriate option for controlling pests and avoiding environmental pollution.

Key words: Aminopolysaccharid, cotton seed coating, antifeedant, germination percentage, lint yield.

INTRODUCTION

China has maintained its position as the largest producer and consumer of cotton in the world (Choudhary and Laroia 2001). However, during the cotton growth process, several types of pest attack cotton delaying its normal growth and development that causes considerable crop losses. The *Aphis gossypii* Glover and *Helicoverpa armigera* Hubner are the most important pests of cotton crops in China. *A. gossypii* feeds on the underside of

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leaves sucking nutrients from the plant. The foliage may become chlorotic and die prematurely. Their feeding also causes a great deal of distortion and leaf curling, hindering photosynthetic capacity of the plant. *H. armigera* prefers to feed on broadleaf species and is a economically important polyphagous pest in China, responsible for considerable damage to cotton.

To prevent large economic losses, seed film-coating technology has been used to reduce the damage of pests. Results are usually positive since the number of pests is reduced and the quality of the seeds is improved when conventional treatments are used. However, these conventional coatings are not the best alternative for the environment due to their accumulative toxicity in the soil (Bautista-Banos *et al.* 2006; Ziani *et al.* 2010). An increasing number of studies have focused on the assessment of ecological risks associated with toxic seed coating agent, with an emphasis on its effects on both target pests and their natural enemies (Joanne 1994; Frutos *et al.* 1999; Men *et al.* 2005).

Due to many problems associated with the use of acute toxic synthetic chemicals as pesticides, a search for an alternate technique for the management of insect pests arises (Isman 1995). Our research showed that the natural aminopolysaccharide extracts from shrimp exhibited a repellent effect against *A. gossypii* and *H. armigera*. Application of the natural aminopolysaccharide in seed film-coating without any adverse effects on agroecosystems is an alternative method. The natural aminopolysaccharide film-coating differs most from traditional ones in which it controls pests through the approach of repelling pests and enhancing the immunity of seeds, but not by killing pests. The main objective of this study was to investigate the effect of coating treatments based on aminopolysaccharide on the pests control, seed germination, cotton growth and yield.

MATERIALS AND METHODS

Materials

Natural aminopolysaccharide (short for APS) extracted from crab was provided from Aokang Biotechnology Company, China. Cotton seeds (Tianrong 2#) were received from Hubei Seed Co., China. Field larval populations were collected from Ezhou of China.

Coating solutions

APS was dissolved at 1%, 2%, 3%, 4% and 5% (w/w) into aqueous solutions of acetic acid at 1% (w/w). The pH of solution was adjusted to 5.0 using 1% NaOH.

Coating process

For APS treatments, a total of 10g of cotton seeds were introduced into a seed coater. During the rotation of the coater, 0.2g solution treatment was added. After coating, the coated seeds were air-dry for two hours at room temperature.

Antifeedant activity test

The antifeedant experiments of the APS were carried out by leaf-disc no choice method (Isman *et al.* 1990). Fresh cotton leaf discs of 4 ×4 cm were punched using cork borer and were dipped in different concentrations of APS solutions. The leaf discs treated with 1% acetic acid and water were used as control. A third-instar larva was introduced into each petri dish. The petri dishes were covered with lid and placed in an incubator at temperature of 26°C and 75% humidity under 16 hours of light ¸8 hours of darkness. Progressive consumption of treated and control leaves area by the larvae after 24 h was recorded using leaf area meter. Ten replications were run for each treatment. The test was valid only the mortality of larvae within 5%. To evaluate the feeding behavior, a "feeding deterrence index" - $FDI_{(\%)}$ was calculated as follow:

$$\boldsymbol{FDI}_{(\%)} = \frac{\boldsymbol{C - T}}{\boldsymbol{T}} \times \mathbf{100}$$

Where C and T represent the amounts eaten of control (water treatment) and treated leaves, respectively.

Germination test

The germination percentage according to International Rules for Seed Testing (1976) is the proportion of normal seeds developed under conditions specified for each seed type. Laboratory cotton seed germination tests were carried out on four randomized replicates of 50 seeds, using petri dishes filled with paddy soil. The dishes were incubated in the constant temperature and humidity incubator at 25±1℃ and air relative humidity of 85%. Seven days after sowing, the percentage of viable plants was recorded. The calculation formulas are as follows:

$$\boldsymbol{GP}_{(\%)} = \frac{\boldsymbol{G_{S7}}}{\boldsymbol{T_S}} \times \mathbf{100}$$

Where $GP_{(\%)}$ is the seed germination percentage, G_{S7} is the number of germinated seeds on the seventh day and T_S is the number of total seeds investigated.

Field trials

This experiment was carried out in Ezhou City, Hubei province, China (30.05°N, 114.31°E). The experiment remained in the same location for two years with no re-randomization of treatments. The experimental design contained a randomized complete block design with four replicates. Each plot was composed of six cotton rows with row spacing of 80 cm and row length of 20m. Cotton was planted on 14 April 2007 and 14 April 2008. With hill-drop planting methods by hand, four seeds per hill were hand dropped into the prepared furrow at in-row plant space of 27.7 cm for 4.5 plants/m^2. In both experiments, cotton in the central rows of each plot was hand-harvested three times at 20-day intervals, lint yield was determined after ginning. The total number of bolls, boll weight and fruit branch number were determined from 20 plants in each plot. Pest counts were made several weeks after planting when they began to emerge (Dong *et al.* 2006).

Statiscal analysis

Since differences in weather data existed between years, results for both experiments in each year were analyzed separately. All the quantitative estimations were analyzed and the values were expressed as mean ± standard error. The data were statistically 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 AND DISCUSSION

Table 1

Consumption of APS-treated cotton leaves by third-instar *A. gossypii* and *H. armigera* over 24 hours

APS Treatment	*Aphis gossypii* Glover		*Helicoverpa armigera* Hubner	
	Mean leaf area eaten [mm^2] ±Standard error	FDI [%]	Mean leaf area eaten [mm^2] ±Standard error	FDI [%]
1%	901.3±65.72b	42.57	1001.2±88.56b	33.01
2%	734.5±59.45c	53.20	976.3±60.81b	34.67
3%	523.9±51.12d	66.62	863.2±61.23c	42.21
4%	310.9±27.34e	80.19	634.2±43.90d	57.56
5%	175.4±11.45e	88.82	238.3±14.56e	84.05
Acetic acid	1490.3±79.23a	-	1467.2±80.97a	-
Water	1569.5±86.87a	-	1494.4±90.10a	-

Antifeedant results

The mean leaf area consumed was significantly less than the control for every APS treatment against *Aphis.* and *H. armigera*. For all APS treatments, leaf area consumed declined with increasing concentration (Table 1).

Germination results

Results related to germination percentage (GP) are shown in Fig. 1. The APS coating treatment resulted in a significant increase in GP as compared to control. The formulation with the highest concentration of APS was the best treatment. However, it was observed that there were not significant differences among 3%, 4% and 5% APS.

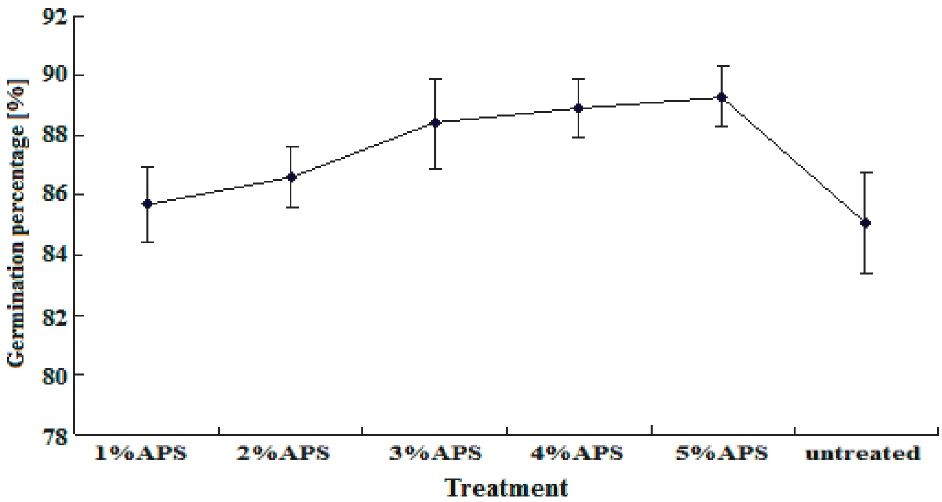

Fig.1. Percentage of germinated seeds treated with different concentration of polysaccharide and the control

Field results

The results indicated that APS coating protect cotton plant against pests efficiently (Table 2). *A. gossypii* and *H. armigera* emergence was significantly reduced on APS-treated cotton seeds especially the high concentration of APS treatment. The main performance indexes of cotton such as the number of bolls, boll weight, number of fruit branches and lint yield were improved under APS treated (Table 3).

Table 2

Suppression of pest emergence by APS coating in fields

Year	Treatment	*Aphis gossypii* Glover emergence [m^{-2}] Weeks after planting		*Helicoverpa armigera* emergence [m^{-2}] Weeks after planting	
		8	16	3	6
2007	1%APS	30.1±0.13	65.4±0.25	59.3±2.89	89.3±2.67
	2% APS	28.1±0.24	51.8±0.18	47.3±1.09	76.3±1.78
	3% APS	20.3±0.18	42.6±0.10	45.3±0.38	65.9±1.28
	4% APS	16.5±0.11	35.3±0.38	38.3±0.38	45.4±1.35
	5% APS	15.3±0.21	28.2±0.19	34.3±0.31	35.9±0.98
	untreated	198.4±6.23	287.8±8.98	189.3±9.36	276.9±7.98
2008	1% APS	55.2±0.12	69.8±1.39	69.4±0.89	79.4±4.01
	2% APS	50.9±0.13	57.8±1.09	60.8±0.87	75.3±3.76
	3% APS	48.7±0.23	50.2±0.98	54.2±0.76	70.3±3.78
	4% APS	36.9±0.34	41.2±0.68	50.4±0.52	68.4±3.98
	5% APS	30.0±0.36	31.3±0.32	40.8±0.38	65.3±4.01
	untreated	219.7±9.34	276.3±8.34	220.3±10.10	290.3±7.90

Table 3

Effects of APS coating on lint yield, yield components of cotton in 2007 and 2008

Year	Treatment	Boll weight [g]	No. of bolls per one plant	No. of fruit branches (per one plant	Lint yield [kg/ha]
2007	1%APS	5.41±0.23	16.0±0.31	13.1±0.32	1285±26.98
	2% APS	5.40±0.12	17.1±0.43	14.6±0.23	1281±24.67
	3% APS	5.30±0.15	17.8±0.34	15.4±0.34	1282±25.87
	4% APS	5.40±0.11	17.9±0.36	16.7±0.31	1299±23.96
	5% APS	5.39±0.10	17.8±0.53	16.8±0.24	1296±24.34
	untreated	5.35±0.14	14.8±0.41	11.2±0.26	1198±21.09
2008	1% APS	5.32±0.19	15.7±0.45	11.6±0.41	1216±20.98
	2% APS	5.38±0.10	16.0±0.34	12.3±0.27	1242±21.34
	3% APS	5.29±0.11	16.3±0.42	12.8±0.38	1243±22.34
	4% APS	5.43±0.12	16.7±0.37	14.0±0.26	1260±25.35
	5% APS	5.37±0.18	17.0±0.44	13.9±0.26	1268±26.33
	untreated	5.27±0.17	14.0±0.34	9.8±0.39	1183±21.35

Mechanism of pest control and yield increase

The APS extracted from shrimp, a biopolymer that has been applied to various area, including agriculture, has been shown to affect many plant responses (Kananont *et al.* 2010). Our research indicated that APS act as antifeedant against *Aphis. gossypii* and *H. armigera*. In the field, use of APS coating would often be in response to larval feeding damage; therefore its performance as a novel seed film-coating would be important in minimizing further damage to the crop. It was known that APS played an important role in the selection of food by insects. The mechanism of the antifeedant activity demonstrated by the APS may be associated with disruption of the physiological processed that this important neuropeptide family regulates in insects. On some larvae, antifeedants can block the stimulant effect of glucose, sacarose and inositol on the cellular chemoreceptors of the sensilla of insect mouth parts. It seems that antifeedant activity of APS would inhibit their ability to form adducts with amine or sulphydril groups on insect receptors (Tokunaga *et al.*, 2004; Perera *et al.*, 2000).As such, APS has a high potential as a source of antifeedant products useful to protect our crops from insects, with interesting perspectives on ecological systems of food production.

There are few published references related to the effect of the APS extracts from shrimp on the crop yield. Bhaskarareddy reported that APS induced resistance to *Fusarium graminearum* and improves wheat seed quality. Barka reported that APS improves development, and protects Vitis vinifera L. against *Botrutis cinerea* (Bhaskarareddy *et al.* 1999; Barka *et al.* 2003). Taken together, these indicate that it is pest damage reduction by APS that is highly correlated with higher crop yield. Another factor that contributes to the cotton yield increase may be due to APS act as a growth stimulator. The active ingredient APS is reported to promote growth and elicit plant defense response in various crops. It increases photosynthesis, promotes and enhances plant growth, stimulates nutrient uptake, increases germination and sprouting, and boosts plant vigor. When used as seed treatment on cotton it elicits an innate immunity response in developing roots which destroy parasitic cyst nematodes without harming beneficial nematodes and organisms (Lu and Wu 2004; Robert *et al.* 2004).

CONCLUSIONS

In summary, the APS demonstrates significant antifeedant activity against *Aphis. Gossypii* and *H. armigera*. Besides, it was shown that APS coating gave significantly better results in terms of germination. In all treatments, APS coating influence positively on the plant growth. It resulted in a strong pest control, in good germination and enhancing crop yield. This result indicated that the use of APS coating allows potential reduction of soil contamination, balance of natural biogeocenose and better economic benefits

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