Efficiency of GCSC-BtA, as a new type of biocide, on different agricultural arthropod pests and its side-effect on some predators

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Abstract: A new type of biocide GCSC-BtA, named from "**G**ermany-**C**hina **S**cientific **C**ooperation-***B****acillus* ***t****huringiensis*-**A**bamectin", was successfully developed by conjugating delta-endotoxin of *Bacillus thuringiensis* (B.t.) with Abamectin from *Streptomyces avermitilis* through conjugator EDC for control of different agricultural arthropod pests. The laboratory results of efficiency of biocide GCSC-BtA to four different arthropod pests and its side-effect on two species of predators are presented in this paper. The results showed significant differences in the mortalities of *Tetranychus cinnabarinus* (BOISD.) (Acari, Tetranychidae), *Frankliniella occidentalis* PERGANDE (Thys., Thripidae), *Aphis fabae* SCOPOLI (Hom., Aphididae) and *Cameraria ohridella* DESCHKA ET DIMIĆ (Lep., Gracillariidae) caused by treatment of 0.6250 mg/ml of formulated product of GCSC-BtA, B.t., Abamectin and Cypermethrin. GCSC-BtA treatment caused higher mortalities of 86.37%, 94.82%, 94.15% and 93.23% in *T. cinnabarinus*, *F. occidentalis*, *A. fabae* and *C. ohridella*, respectively. Mortalities caused by Abamectin treatment were 73.92%, 63.3%, 86.52% and 73.48%, and by Cypermethrin treatment were 44.29%, 57.28%, 97.68% and 51.26%, respectively. B.t. could only kill *C. ohridella* with 87.62% mortality. Pesticidal treatment gave variations in mortalities of *Orius strigicollis* POPPIUS (Het., Anthocoridae) and *Stethorus cantonensis* PANG (Col., Coccinellidae) predators at $p < 0.01$, where 0.6250 mg/ml GCSC-BtA caused 21.76% and 32.42% mortalities, while the same rate of products of Abamectin with 73.26% and 87.67% as well as Cypermethrin with 86.43% and 93.83% mortalities, respectively. It is concluded that the biocide GCSC-BtA has increased efficacies as well as host ranges of B.t. and Abamectin, but displayed safer to some predators.

Key words: GCSC-BtA, *Tetranychus cinnabarinus*, *Frankliniella occidentalis*, *Aphis fabae*, *Cameraria ohridella*, *Orius strigicollis*, *Stethorus cantonensis*

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In agricultural ecosystem, there exist many arthropod pests from different orders such as Acari, Thysanoptera, Homoptera and Lepidoptera (TANG 1992, RODRIGUEZ et al. 1997, TOMICZEK & KREHAN 1998, DEML et al. 1999), simultaneously companying with predators from the orders of Heteroptera and Coleoptera (ZEIRUK et al. 2002). It has been a normal practice to control arthropod pest outbreaks by pesticidal application (VIKRAM et al. 2000), which often results in situation without pests suppression, rather insecticide resistance and pest-enemy balance destruction (WORKMAN et al. 1980, SHARMA & KASHYAP 2002). *Bacillus thuringiensis* (B.t.) was one of the highly recommended biocides to be used for pest suppression in the agricultural ecosystem, but its efficacy was limited by low killing speed as well as narrow host range (LIU & SENGONCA 2002). A new idea of multiple-toxin was worked out to conjugate the B.t. toxin with Abamectin from *Streptomyces avermitilis* through conjugator EDC to form a new type of biocide, which was named GCSC-BtA (**G**ermany-**C**hina **S**cientific **C**ooperation-***B****acillus* ***t****huringiensis*-**A**bamectin). The new biocide GCSC-BtA has been proved with higher pest control efficacy than B.t. and Abamectin alone against a wide range of agricultural pests (SENGONCA et al. 2001).

The present research aimed to investigate efficiency of GCSC-BtA against different agricultural pests, i.e., *Tetranychus cinnabarinus* (BOISD.) (Acari, Tetranychidae), *Frankliniella occidentalis* PERGANDE (Thys., Thripidae), *Aphis fabae* SCOPOLI (Hom., Aphididae), and the horse-chestnut leafminer *Cameraria ohridella* DESCHKA ET DIMIĆ (Lep., Gracillariidae), as well as its side-effect on two predators *Orius strigicollis* POPPIUS

(Het., Anthocoridae) and *Stethorus cantonensis* PANG (Col., Coccinellidae), in comparison with B.t. or Abamectin alone as well as a commercial insecticide Cypermethrin.

Materials and Methods

The present experiments were carried out in both laboratories in Institute of Phytopathology, University of Bonn, Germany and Biotechnology Center, Fujian Academy of Agricultural Sciences, PR China. For the experiments the immature stages of *F. occidentalis* and *C. ohridella* were obtained from stock cultures of the Institute. *T. cinnabarinus* and *A. fabae* as well as the predators *O. strigicollis* and *S. cantonensis* were reared in climate chambers at 25 ± 1°C temperature, 75 ± 10% RH and a 12:12 h (L:D) photoperiod in the Biotechnology Center.

In bioassay procedure, the following treatments were included, e.g. the self-prepared biocide GCSC-BtA according to LIU & SENGONCA (2003), 10 ml of 10 mg/ml B.t. protoxin was mixed with 10 ml of 20 mg/ml Abamectin-COONa, and then 383.4 mg fresh conjugator EDC was added to obtain a final concentration of 0.1 M. The bioconjugation system was kept at about 25°C room temperature for 24 h. The bioconjugated substance from the final reaction was named as GCSC-BtA, which consisted of 15 mg/ml active ingredient with equivalents to 5 mg/ml B.t. protoxin and 10 mg/ml Abamectin), self-prepared B.t. crystal (B.t. var. *kurstaki* LSZ9408, purify>95%) and commercially available Abamectin (1.8% EC, Hebei Vian Pesticide Co., Ltd., China), and Cypermethrin (2.5% EC, Monsanto, USA). The insecticide-residue bioassay method used was modified after CONSOLI et al. (1995). Each control agent was diluted in water to obtain serial concentrations of 0.1563, 0.3125, 0.6250, 1.2500 and 2.5000 mg/ml of formulated product for LC_{50} test. The host plant cabbage leaves were immersed in the insecticide solutions for 5 sec. Afterwards, they were dried in the air and placed in Petri dishes (9 cm in diameter and 2 cm in height) with moist cotton attaching to the end of leaves. Thirty individuals of the target arthropods were transferred to each separate dish. Also 30 individuals of the predators were exposed at the same way into Petri dishes with the immature stages of *A. fabae* as prey. Each insecticidal concentration was replicated three times. For *C. ohridella* experiments, small fresh horse-chestnut branches were cut with pest's infestation and 30 individuals of 4th instar larva on leaves were marked with pen. And then the leaves were dipped into the insecticide concentrations for 5 sec and air dried. The branches were put in bottles with water in the climate chambers. Also there were three replications for each treatment. The leaves were only immersed in tap water as control. Pest mortalities were recorded 72 h after treatments. All the experiments were conducted at 25 ± 1°C temperature, 75 ± 10% RH and a 12:12 h (L:D) photoperiod.

The results of mortality data at different concentrations were subjected to probit analysis of LC_{50} and average mortality was determined for comparison at the 1% level (TUKEY's HSD) Multiple Range Test using DPS® 1.0 software of windows 98 (TANG 1998).

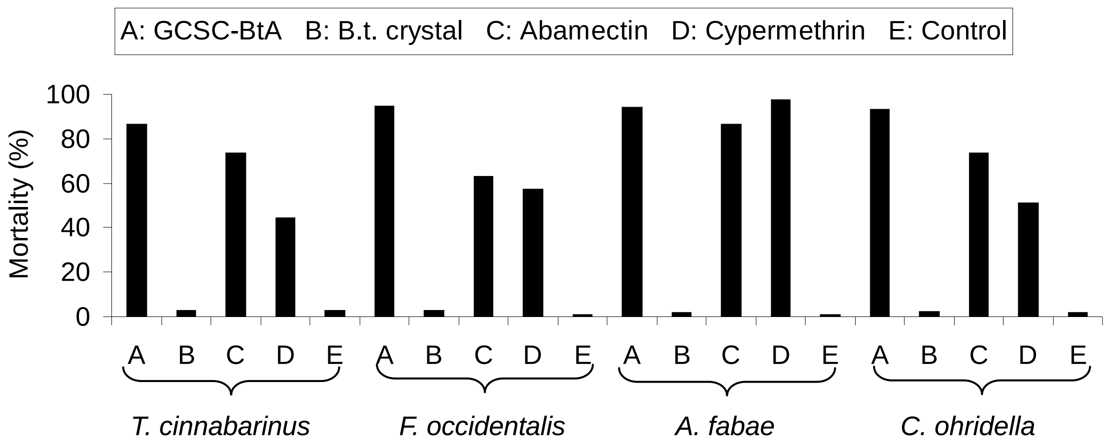

Fig. 1: Percentage mortalities of the tested pests *Tetranychus cinnabarinus*, *Frankliniella occidentalis*, *Aphis fabae* and *Cameraria ohridella* caused by GCSC-BtA, B.t. crystal, Abamectin, Cypermethrin and water control at a concentration of 0.6250 mg/ml product after 72 h.

Results

Mortality and LC_{50} values of the tested pests

The results showed significant differences in mortalities of *T. cinnabarinus*, *F. occidentalis*, *A. fabae* and *C. ohridella* caused by GCSC-BtA, B.t. crystal, Abamectin and Cypermethrin at 1% level (Tukey's HSD) Multiple Range Test. GCSC-BtA caused highest pest mortalities of 86.37%, 94.82%, 94.15% and 93.23% of *T. cinnabarinus*, *F. occidentalis*, *A. fabae* and *C. ohridella*, respectively (Fig. 1). Pest mortalities caused by Abamectin treatment were 73.92%, 63.3%, 86.52% and 73.48%, while by Cypermethrin 44.29%, 57.28%, 97.68% and 51.26% in the aforementioned pests, respectively. B.t. crystal didn't show obvious lethal effects to these four pests.

The LC_{50}s of GCSC-BtA, B.t.crystal, Abamectin and Cypermethrin for *T. cinnabarinus*, *F. occidentalis*, *A. fabae* and *C. ohridella* are presented in Table 1. It is clear from the results that LC_{50}s of GCSC-BtA to all the pests are lower than of the other control agents, expect to that of Cypermethrin to *A. fabae*.

Tab. 1: Susceptibilities (LC_{50}) of *Tetranychus cinnabarinus*, *Frankliniella occidentalis*, *Aphis fabae* and *Cameraria ohridella* pests to GCSC-BtA, B.t. crystal, Abamectin and Cypermethrin after 72 h at 25 ± 1°C

Arthropod pests	Order	Control agent	LC_{50} (95%CL) (mg product/ml)	Slope	SE
Tetranychus cinnabarinus	Acari	GCSC-BtA	0.0025 (0.0022~0.0028)	4.3513	0.5498
		B.t.	> 10		
		Abamectin	0.0015 (0.0010~0.0019)	6.1932	0.9767
		Cypermethrin	1.1820 (0.0849~2.4240)	1.0983	0.1590
Frankliniella occidentalis	Thysanoptera	GCSC-BtA	0.0027 (0.0017~0.0034)	3.4123	0.4882
		B.t.	> 10		
		Abamectin	0.2823 (0.1800~0.3764)	2.5003	0.3866
		Cypermethrin	1.0296 (0.7243~1.5925)	1.6515	0.2372
Aphis fabae	Homoptera	GCSC-BtA	0.3999 (0.2730~0.6402)	1.4148	0.2381
		B.t.	> 10		
		Abamectin	0.8781 (0.0765~2.2882)	0.5328	0.1930
		Cypermethrin	0.0112 (0.0087~0.0143)	2.2002	0.3288
Cameraria ohridella	Lepidoptera	GCSC-BtA	0.0240 (0.0098~0.0400)	1.2137	0.2520
		B.t.	> 10	1.9031	0.1452
		Abamectin	0.2703 (0.0032~0.8120)	0.5310	0.2156
		Cypermethrin	1.1651 (0.3420~1.5067)	0.4629	0.1705

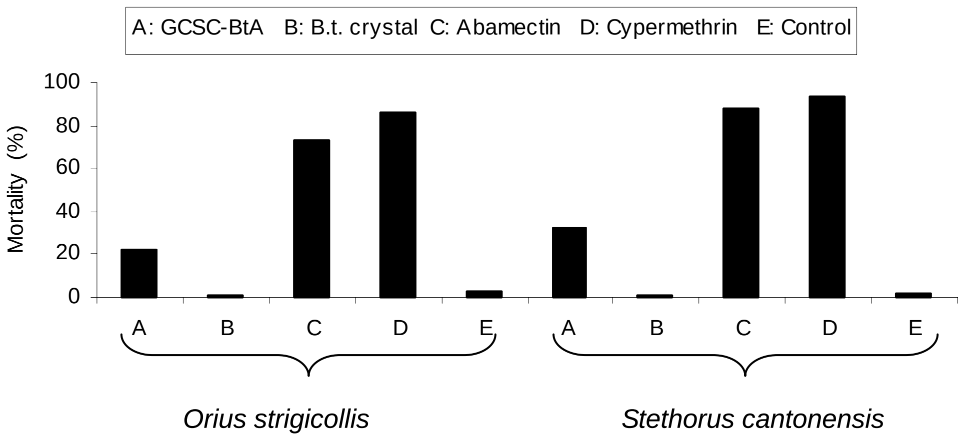

Fig. 2: Percentage mortalities of tested predators *Orius strigicollis* and *Stethorus cantonensis* caused by GCSC-BtA, B.t. crystal, Abamectin, Cypermethrin and water control with a concentration of 0.6250 mg product /ml after 72 h.

Mortality and LC_{50} values of the tested predators

The results of this experiment showed significant differences in mortalities of *O. strigicollis* and *S. cantonensis* caused by GCSC-BtA, B.t. crystal, Abamectin and Cypermethrin at 1% level (Tukey's HSD) Multiple Range Test. GCSC-BtA caused 21.76% and 32.42% mortalities of *O. strigicollis* and *S. cantonensis*, while Abamectin 73.26% and 87.67%, and Cypermethrin 86.43% and 93.83%, respectively (Fig. 2).

Table 2 shows LC_{50} values of GCSC-BtA, B.t., Abamectin and Cypermethrin for *O. strigicollis* and *S. cantonensis*. It can be seen from the results that LC_{50} values of GCSC-BtA for the two predator species were higher than the other control agents except B.t. crystal, because B.t. didn't have efficiency to insects out of Lepidoptera.

Tab. 2: Susceptibilities (LC_{50}) of *Orius strigicollis* and *Stethorus cantonensis* predators to GCSC-BtA, B.t. crystal, Abamectin and Cypermethrin after 72 h at 25 ± 1°C

Predator	Order	Control agent	LC_{50} (95%CL) (mg/ml)	Slope	SE
Orius strigicollis	Heteroptera	GCSC-BtA	1.9018 (0.3944~2.3432)	0.5827	0.0693
		B.t. crystal	>10		
		Abamectin	0.7334 (0.4650~1.2672)	0.6361	0.0539
		Cypermethrin	0.0294 (0.0039~0.1244)	0.6436	0.0584
Stethorus cantonensis	Coleoptera	GCSC-BtA	3.0192 (1.6502~6.6002)	0.5776	0.0521
		B.t. crystal	>10		
		Abamectin	0.1681 (0.1085~0.2590)	0.6130	0.0448
		Cypermethrin	0.0952 (0.0233~0.3768)	0.7398	0.0564

Discussion

A new type of biocide GCSC-BtA was successfully developed by conjugating delta-endotoxin of *Bacillus thuringiensis* with Abamectin from *Streptomyces avermitilis* through conjugator EDC for the control of agricultural arthropod pests (SENGONCA et al. 2001). Agreeing with the conclusion done by SENGONCA et al. 2001 and LIU & SENGONCA 2002, in the present experiments the biocide GCSC-BtA demonstrated higher efficiency to control the agricultural pests *T. cinnabarinus*, *F. occidentalis*, *A. fabae* and *C. ohridella* from different arthropod orders in the laboratory than B.t. crystal or Abamectin alone as well as an insecticide Cypermethrin. On the other hand, it was less toxic to the *O. strigicollis* and *S. cantonensis* predators than Abamectin or Cypermethrin. It is concluded that the biocide GCSC-BtA has increased efficacy as well as widened the control host range of the B.t. or Abamectin alone and has proved to be safer to the two predators species than Abamectin and Cypermethrin used at the same rate of product in the laboratory situation.

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