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Toxicities of destruxins against *Bemisia tabaci* and its natural enemy, *Serangium japonicum*

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ABSTRACT

The bioactivities of destruxins against whitefly, *Bemisia tabaci* and its natural enemy, ladybird beetle *Serangium japonicum* were evaluated. Destruxins A and B (DA and DB) showed insignificant ovicidal, oviposition deterrent and systemic insecticidal activities to *B. tabaci*; however, DA and DB had certain contact virulence to its nymphs. The LC_{50} values of DA at 120 h to 2nd, 3rd and 4th instars were 89.8 (95% confidence interval as 85.4–94.4), 199.3 (187.7–211.5) and 270.7 (251.5–291.5) mg/L, while the LC_{50}s of DB at 120 h were 96.5 (92.0–101.2), 216.7 (203.0–231.2), 359.4 (326.6–395.4) mg/L, respectively. In addition, DA exhibited moderate acute contact toxicities towards *S. japonicum*, the LC_{50}s at 48 h were 165.4 (132.3–229.4) and 192.5 (148.1–289.2) mg/L for 4th instar larvae and adults. Furthermore, the results from experiments of residual toxicities of DA towards mortalities of 4th instar larvae and adults, pupation rate, emergence rate, average number of egg/female and hatching rate suggested that DA had minimal effects to the ladybird beetle. Generally, the toxicity decreased about 50% from 1st to 3rd–5th day of post-treatment. Specially, the residual toxicity at 50 mg/L and the 7th day post-treatment was down to a value not differing significantly from the control.

1. Introduction

Some entomopathogenic fungi such as *Metarhizium anisopliae* and *Aschersonia* spp. can produce various kinds of mycotoxins, i.e. destruxin (Krasnoff et al., 1996; Starsser et al., 2000). Destruxins are typically composed of five amino acids and an α-hydroxy acid forming a cyclic hexadepsipeptide whereas 35 destruxin analogues have been reported to date. Destruxins have multi-bioactive characteristics such as insecticidal, herbicidal and antiviral, etc. (Pedras et al., 2002; Hu and Ren, 2004). The insecticidal activities of destruxins have been described in a number of documents and generally, the LC_{50}s values were on the level as $10–100 \times 10^{-6}$ mg/L (Poprawski et al., 1994; Amiri et al., 1999; Thomsen and Eilenberg, 2000). But, a recent research reported that destruxin achieved a very exciting result against 12-day-old larvae of *Spodoptera litura*. The value of LD_{50} was as low as 0.045 μg/g body weight in the combined application assay compared with the corresponding values of 0.17 μg/g body weight in the ingestion assay and 0.237 μg/g body weight in the topical application (Sree et al., 2008).

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) biotype B is a worldwide pest of many ornamental, agricultural and greenhouse crops (Naranjo and Ellsworth, 2001). In China, *B. tabaci* became an important agricultural pest during late 1990s (Ren et al., 2001). This pest has very strong adaptation to hosts (An et al., 2007) and can rapidly develop resistance to insecticides; therefore biocontrol is emerging as a necessary method to control it (Ren et al., 2001).

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The ladybird beetle, *Serangium japonicum* (Coleoptera: Coccinellidae) is an important natural enemy of whitefly in China (Yao et al., 2005). The beetle usually concurs with the other effective control factors of natural population, e.g., the pathogenic fungi, *Aschersonia* spp. (Qiu et al., 2003; Musa and Ren, 2005). However, *Aschersonia* species can produce destruxins (Krasnoff et al., 1996).

To date, hundreds of articles related to destruxins have been published, but these focused only on the action mechanisms, bioactivity, detection and production of destruxins. Nevertheless, the influence of destruxins on natural enemies of pests is overlooked. In this study, the objectives were paid attention to: (a) the ovicidal, oviposition deterrent, systemic and contact activities of DA and DB against *B. tabaci*; (b) the toxic effects of destruxin against the beneficial natural enemy insect *S. japonicum*.

2. Materials and methods

2.1. Insects

B. tabaci individuals were maintained at $25 \pm 0.5\,^{\circ}C$, $70 \pm 5\%$ RH and 16:8 (L:D) photoperiod in growth cages ($60 \times 60 \times 60$ cm) on potted-plants of *Hibiscus rosasinensis* L. for egg laying. On appropriate development 2nd, 3rd and 4th instars nymphs were used for bioassay. The 4th instars larvae and adults of *S. japonicum* were taken from the reared population of department of entomology, South China Agricultural University (SCAU).

2.2. Destruxins

Destruxins A and B were isolated and purified according to Pais et al. (1981) and Hu et al. (2006). The stock solutions of DA and DB were prepared as 100,000 mg/L with (acetone/acetoacetate = 1/1) and stored at $-20\,^{\circ}C$ for further use. The stocks were diluted with 0.05% Tween 80 into working solutions for the bioassay.

2.3. Bioactivities against B. tabaci

2.3.1. Ovicidal activity

The methods of Xu et al. (2003) were referred. The *H. rosasinensis* leaves with 1-day-old eggs (at least 200 eggs/leaf) of *B. tabaci* were immersed in different concentrations (1200, 600, 300, 150, 75 mg/L) of DA and DB for 10 s. The control was only immersed in the solution of 0.05% Tween 80. The immersed leaves were kept for drying at room temperature. After dryness, these leaves were pasted on the agar plates (agar 15 g, water 1000 mL). The leaf stalk was wrapped with wet cotton pad for preventing it from drying. The agar plates with the treated eggs were kept in the climatizer with $25 \pm 0.5\,^{\circ}C$, $70 \pm 5\%$ RH and photoperiod of 16:8 (L:D). Each treatment was replicated three times and entire assay was repeated twice. The data of eggs mortality was recorded after seven days.

2.3.2. Oviposition deterrent activity

The stalks with new leaves of *H. rosasinensis* were taken from the potted plant. The leaves were dipped in different concentrations as described above. For control treatment the leaves were only immersed in 0.05% Tween 80 solutions. After drying, the stalked leaves were inserted in a block of floral foam placed in a Petri dish with water. These stalked leaves were numbered and placed in the growth cage. Fifteen pairs of adults of *B. tabaci* were introduced per leaf. There were five treatments 1200, 600, 300, 150, 75 mg/L of DA and each treatment was replicated thrice. The experiment was repeated twice. The oviposition deterrent data was recorded after 24 h of treatment. The rates of oviposition deterrent were evaluated according to Li et al. (2005).

2.3.3. Systemic insecticidal toxicity

Leaf stalk wrapped with cotton pad (Wu et al., 2003) was used to test the systemic insecticidal activities. The potted-plants with 2nd, 3rd and 4th instar nymphs were used. The cotton pads were attached at the basal part of the stalk of potted plant, then the cotton pads were soaked with 0.1 mL DA or DB of different concentrations (1000, 100 and 10 mg/L). Control treatment was treated with the same procedure but with 0.05% Tween 80 solution. The culture conditions were same as that of 2.1. The experiment was repeated twice with three replicates in each treatment (three potted-plants/treatment). The results of mortality of different nymphal instars were recorded after 48, 96 and 144 h treatment.

2.3.4. Contact virulence

Leaf dip method was used to examine the contact toxicity of DA and DB against 2nd, 3rd and 4th instar nymphs. The leaves with 2nd, 3rd and 4th nymphs were detached from the plants and were treated as described in Section 2.3.1. The stock solution was diluted to the working concentration series (600, 300, 150, 75, 37.5, 18.75, 9.38 mg/L). An area with more than 100 nymphs in each leaf was marked so as to record the data. Three replications were used per treatment and the experiment was repeated twice. The mortality data was recorded before treatment and after 8, 16, 24, 48, 72, 96 and 120 h of the treatment. The complementary log–log (CLL) and time dose mortality (TDM) model (Preisler and Robertson, 1989) were used for data analysis. DPS software (Data Processing System, Version 3.01, China) (Tang and Feng, 2002) was employed to analyze the data.

2.4. Toxicity to S. japonicum

2.4.1. Contact toxicity

The method of Wang and Shen (2002) was referred. DA stock solution was diluted with acetone to the serial working solutions of 300, 150, 75, 37.5 and 18.75 mg/L. The 4th instar larvae and 10 days old adults of ladybird beetles were used for bioassay. From each concentration 0.1 ml working solution was poured in a test tube. The test tubes were turned so that the solution could spread evenly. After evaporation of the solvent, one individual of *S. japonicum* was introduced into a tube and the tube was closed with cotton pad and cultured at the same conditions as described above. Ten larvae or adults were used in each treatment with three replications. The same experiment was repeated twice. The mortalities at 24 and 48 h were

investigated. The LC-p equations and LC_{50}s were evaluated by means of DPS software.

2.4.2. Residual toxicity

The method of Ma et al. (2006) was referred. The potted-plants of *H. rosasinensis* with *B. tabaci* individuals were sprayed with DA working solutions of 300, 150 and 50 mg/L till the leaves were completely wetted. The treated plants were maintained in greenhouse at about 25 °C, 70% RH and 12:12 (L: D) photoperiod. Fourth instar larvae and 10 days old adults of ladybird beetles were introduced on the leaves on the 1st, 3rd, 5th and 7th day post-treatment of DA and covered with a nylon cage per pot-plant. Ten larvae or five couples of adults were used in each treatment with three replications. The same experiment was repeated twice. The mortalities were recorded at 24 and 48 h after the exposure of beetles whereas the pupae or egg numbers at 7th day and emergence or hatching rate (%) at 14th day after the exposure were investigated. The means were subjected to analysis of variance (ANOVA) with DPS software.

3. Results

3.1. Bioactivities against B. tabaci

3.1.1. Ovicidal activity

The data revealed that there was no statistically significant ($P > 0.05$) difference between the destruxins and the control treatment. The mortality percentages were recorded 17.67 ± 1.45 and 18.33 ± 0.88 for DA and DB after 7 days of the treatment at a concentration of 1200 mg/L, and in contrast to this, control had a mortality percentage of 9.67 ± 1.45 that indicates that DA and DB have no ovicidal activity against *B. tabaci*.

3.1.2. Oviposition deterrent activity

The results indicated that DA had insignificant ($P > 0.05$) oviposition deterrent activity, the data reveals that even at high concentration of 1200 mg/L, the deterrent rate (%) was only 4.91 ± 2.26 which has no significant difference from other treatments and control.

Table 1
Contact virulence of DA and DB against *B. tabaci* nymphs.

Nymph	Time post-treatment (h)	LC_{50} (95% confidence limits, CL) (mg/L)	
		DA	DB
2nd	24	168.1 (155.9–181.2)	174.1 (162.0–187.1)
	48	121.3 (114.3–128.8)	137.4 (129.5–145.8)
	72	97.2 (92.3–102.4)	111.6 (106.1–117.5)
	96	94.4 (89.6–99.3)	105.0 (99.9–110.3)
	120	89.8 (85.4–94.4)	96.5 (92.0–101.2)
3rd	24	320.4 (294.8–348.2)	613.1 (539.9–696.3)
	48	216.0 (202.9–230.0)	292.6 (270.1–317.1)
	72	211.1 (198.4–224.5)	243.4 (226.9–261.1)
	96	205.9 (193.8–218.9)	224.7 (210.2–240.1)
	120	199.3 (187.7–211.5)	216.7 (203.0–231.2)
4th	24	327.2 (299.9–356.9)	415.9 (374.0–462.5)
	48	297.9 (275.0–322.7)	375.7 (340.2–414.9)
	72	271.4 (252.0–292.2)	367.9 (333.7–405.5)
	96	270.8 (251.5–291.6)	359.4 (326.7–395.5)
	120	270.7 (251.5–291.5)	359.4 (326.6–395.4)

Table 2
The LT_{50}s of DA and DB against *B. tabaci* in contact virulence.

Nymph	Concentration (mg/L)	LT_{50} (h)	
		DA	DB
2nd	150.00	32.1	32.7
3rd	300.00	27.9	47.1
4th	300.00	46.2	

3.1.3. Systemic insecticidal activity

The data revealed statistically insignificant difference between different concentrations and the control. For 2nd instar nymph of whitefly, the mortalities (%) of DA and DB at 1000 mg/L after 48 h were 2.96 ± 0.15 and 3.30 ± 0.19 with the CK value 2.74 ± 0.16. Even at 144 h post-treatment only a minute increase in mortality percentage was recorded.

For the 3rd instar nymphs, 48 h post-treatment, the mortality percentages of 2.98 ± 0.13 and 2.68 ± 0.15 were recorded at 1000 mg/L of DA and DB. No sharp increase in mortality percentage was observed at 96 and 144 h.

3.1.4. Contact virulence against nymph

The destruxins had a considerable impact at different concentrations and durations. In general, the toxicities increased as the concentrations and time increased and the stage of the nymphs decreased. According to the CLL modeling, the LC_{50} values were yielded (Table 1). The results showed that the LC_{50} values of different nymphal instars were time dependent. For second instar nymphs, DA had the LC_{50} values 168.1 (155.9–181.2) and 89.80 (85.4–94.4) mg/L at 24 and 120 h post-treatment, meanwhile DB had LC_{50} values of 174.1 (162.0–187.1) and 96.5 (92.0–101.1).

On the other hand, for the 3rd instar nymphs, the LC_{50} for DA and DB reached up to 320.4 (294.8–348.2) and 613.1 (539.9–696.3) mg/L at 24 h and 199.3 (187.7–211.5) and 216.7 (203.0–231.2) mg/L at 120 h after treatment. In contrast to this for the 4th nymphal instar, DA and DB showed the least effectiveness with the LC_{50} of 327.2, 415.9 mg/L and 270.7, 359.4 mg/L at 24 and 120 h, respectively.

The LT_{50} data showed that the destruxins had a slower effectiveness (Table 2), especially to older nymphs. The LT_{50} values for the 2nd instar nymph were 32.1 h and 32.7 h at 150 mg/L of DA and DB. For the 3rd instar, the LT_{50} for DA and DB at 300 mg/L were 27.9 and 47.1 h. However towards 4th instar nymph, DA at a concentration rate of 300 mg/L gave the LT_{50} value of 46.2 h and for DB it could not be evaluated as it needed a concentration more than 300 mg/L.

Table 3
LC_{50}s of DA against *S. japonicum*.

Insect stage	$LC_{50}$and 95% CL (mg/L)	
	24 h	48 h
4th Instar larvae	200.4 (153.5–306.9)	165.4 (132.3–229.4)
Adults	212.2 (159.2–337.7)	192.5 (148.1–289.2)

3.2. Toxicity to S. japonicum

3.2.1. Contact toxicity

The LC_{50} values indicated that DA had moderate toxicities towards the ladybird beetle (Table 3). The 4th instar larvae were proved to have similar susceptibility as adults. The LC_{50} values were 200.4 (153.5–306.9) and 212.2 (159.2–337.7) at 24 h, and 165.4 (132.3–229.4), 192.5 (148.1–289.2) at 48 h after treatment, respectively.

3.2.2. Residual toxicity

For 4th instar larvae of *S. japonicum*, mortalities, pupation and emergence rates changed significantly with the days after DA was sprayed at different concentrations and the larvae were introduced (Fig. 1). At 300 mg/L of DA, the percent mortality at 24 and 48 h (Fig. 1A, B) were recorded as 52.5 and 70.0, 35.9 and 59.0, 17.50 and 35.0, 10.00 and 15.0; the pupation rate (%) were 20.0, 28.2, 56.4 and 80.0 (Fig. 1C) and the adult emergence rates showed a percentage of 17.5, 28.2, 53.9 and 75.0 (Fig. 1D) on 1st, 3rd, 5th, 7th day of treatment, respectively. However, at 50 mg/L, the percent mortality at 24 h decreased accordingly to 22.5, 10.3, 5.0 and 0.0 (Fig. 1A); the pupation and emergence percent rate (Fig. 1C, D) increased to 65.0 and 60.0, 77.5 and 77.5, 90.8 and 87.5, and 100.0 and 95.0. In general, the toxicity decreased about 50% from 1st to 3rd–5th day of treatment. Specially, the residual toxicity at 50 mg/L and the 7th day post-treatment was down to a value not differing significantly from the control.

In case of adults of *S. japonicum* almost similar results were obtained as that of larval treatment with DA (Fig. 2). Generally, the toxicity of DA decreased significantly from 3rd day of treatment and decreased about 50% from 3rd to 5th day. At 300 mg/L, the mortality percentages after 24 h were recorded as 59.5, 49.0, 21.3 and 11.0; average number of egg/female was 12.5, 13.9, 20.7 and 37.7, while the hatching percentage was 92.0, 90.4, 94.5 and 93.6 on 1st, 3rd, 5th, 7th day of post-treatment, respectively. However,

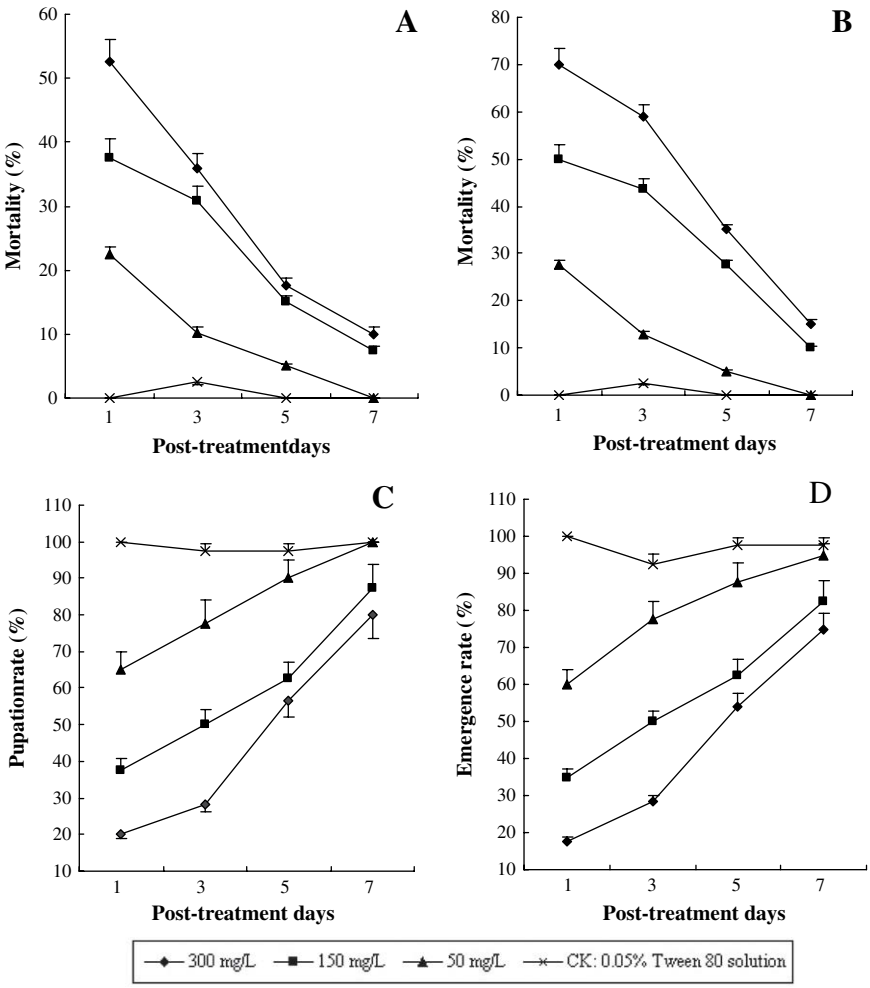

Fig. 1. The changes in mortality, pupation rate and adult emergence of *S. japonicum* after its 4th instar larvae were exposed on the potted-plants of *H. rosasinensis* with *B. tabaci* individuals sprayed with DA. The *X*-axis was post-treatment days, i.e. the days after DA were sprayed and the larvae were introduced. (A) mortality percents of 4th instar larvae after 24 h exposure. (B) Mortality percents of 4th instar larvae after 48 h exposure. (C) Pupation rate (%) of 4th instar larvae after 7 day exposure. (D) Percent adult emergence of 4th instar larvae after 14 day exposure.

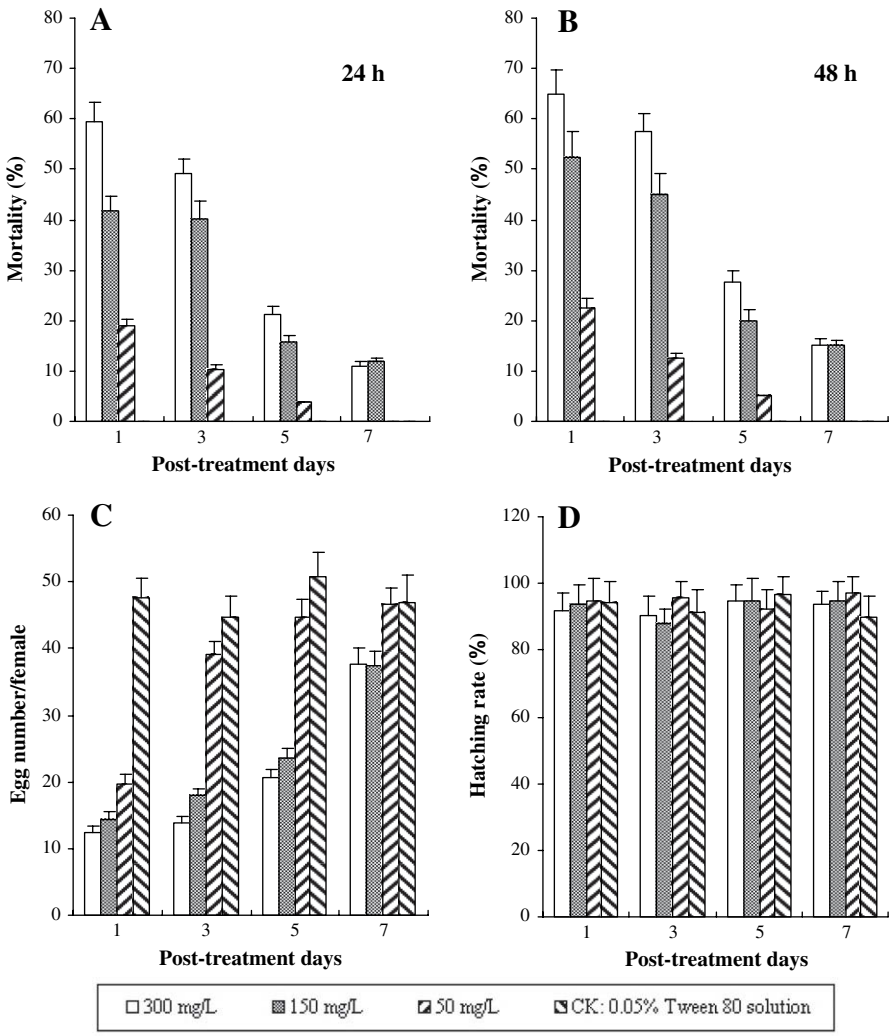

Fig. 2. The changes in mortality, fecundity and hatching percentage of *S. japonicum* after its adults were exposed on the potted-plants of *H. rosasinensis* with *B. tabaci* individuals sprayed with DA. The *X*-axis was post-treatment days, i.e. the days after DA were sprayed and the larvae were introduced. (A) Mortality percents of adult beetles after 24 h exposure. (B) Mortality percents of adult beetles after 48 h exposure. (C) Average fecundity of each female *S. japonicum* after 7 day exposure. (D) Hatching percentage of eggs laid by the surviving females after 14 day exposure.

at 50 mg/L, the mortalities (%), average number of egg/female and hatching percentage were recorded as 19.0, 10.3, 3.8 and 0.0, 19.6, 39.1, 44.7 and 46.6, and 94.6, 95.6, 92.6, 97.0, respectively.

The DA also exhibited little effectiveness to the pupation, emergence, ovipositioning and hatching ability of surviving beetles.

4. Discussion

Bioactivity of destruxins to various insects has already been reported, having different mode of actions such as contact, antifeedant and growth regulation. These reports did not include systemic, oviposition and deterrent insecticidal activities (Pedras et al., 2002; Hu and Ren, 2004). In this research, the comprehensive bioassays of destruxins against whitefly are reported first time. The results revealed that there were either no or minute ovicidal, oviposition deterrent and systemic insecticidal activities of destruxins, but a significant contact toxicity to whiteflies was observed. This knowledge can be helpful to explore the toxicological mechanism of destruxins. As toxicity of chemicals is usually contributed to the interaction between chemicals and their target protein, it is suggested that the reason of non-ovicidal and non-oviposition deterrent toxicity of destruxins is perhaps because of the absence of target proteins in eggs and corresponding organs or tissues in relation to behavior of oviposition. However, no systemic insecticidal activities by destruxin might be due to its non-transportation by plant. Of course, the research results supply enough and encouraging information for application of destruxins to control whitefly.

With regards to the contact virulence, different LC_{50} values of destruxins were reported in respective bioassay researches. Generally, the LC_{50} values were on the level as $(10–100) \times 10^{-6}$ mg/L (Poprawski et al., 1994; Amiri et al.,

1999; Thomsen and Eilenberg, 2000). The result in this study gave the similar level of LC_{50} values. However, a recent research revealed a very exciting LD_{50} of destruxins against 12-day-old larvae of *S. litura*. The value of LD_{50} was as low as 0.045 μg/g body weight in the combined application assay, 0.17 μg/g body weight in the ingestion assay and 0.237 μg/g body weight in the topical application (Sree et al., 2008). It implies that the method of application influences the toxicity of destruxins. But whitefly is a tiny insect absorbing host plant sap, ingestion and topical application are unavailable. Maybe, more suitable and accurate bioassay methods are required to develop.

The LT_{50} values of destruxins were determined by Thomsen and Eilenberg (2000) with the modeling CLL technology and observed that *Pieris brassicae* showed a quicker (1–2 days) susceptibility while *Agrotis segetum* proved to be more resistant (10 days) towards destruxins. In our experiment, whitefly's younger nymphs showed a quicker effectiveness similar to *P. brassicae*, the possible reason might be that whitefly has distinctive biological characteristics such as the tiny body and shorter life span. Of course, the ability of penetrating cuticle should be noticed as well.

Although destruxins are not as efficient as some chemical insecticides such as neonicotinoids against whitefly, however, because destruxin is a kind of immunosuppressive agent aiming at hemolymph (Vey et al., 2002; Vilcinskas et al., 1997), synergistic interaction between destruxin and other insecticides should be paid close attention. In fact, the synergism of destruxins with Bt (Brousseua et al., 1998) and *Paecilomyces javanicus* (Hu et al., 2007) had been observed. As far as it is concerned that destruxins have contact virulence to 2nd and 3rd nymphal whitefly, they should be taken into account for further study in aspects of synergisms with synthetic and microorganic insecticides to control whitefly.

There have been little reports about toxicity of destruxins to beneficial insects. In this experiment, although DA showed certain contact toxicity to *S. japonicum*, however, its residual toxicity decreased 50% on the 3rd–5th days post-treatment. The larvae and adults were able to survive from infection of DA and they pupate, emerged, laid eggs and subsequently hatched normally.

The toxicity of DA against *S. japonicum* is related closely to the property of DA, ladybird beetle, whitefly, plant and environments. At first, detoxification and transformation of destruxins in environments (ladybird beetle, whitefly and plant) are very important. In fact, destruxins are unstable in environments; they are not only decomposed by light but also biodegraded and biotransformed by different lives (Pedras et al., 2003; Dudley et al., 2004). Of course, to some extent, whitefly, lady beetle and *H. rosasinensis* as well can detoxify destruxins, but how they degrade destruxins is unknown. However, whitefly as a pest possessing very strong adaptation to hosts and foods is known to all (Ren et al., 2001; An et al., 2007), it means that whitefly has smart ability to detoxify toxicants in foods. Secondly, plant acts an important role as well, beside directly degrade and transform DA, the absorption of plants to destruxin could decrease the dosage of insects contact and the growth of host plant could enlarge the new leaves area that supply safe place to lady beetles. Therefore, based on the above consideration, it is concluded that the toxicity of DA to lady beetle might be insignificant, especially in fields.

However, destruxins can be produced by the very important pathogenic fungi of whitefly, *Aschersonia* spp. (Krasnoff et al., 1996; Pedras et al., 2002). So, the interaction between *Aschersonia* and *S. japonicum* becomes another area of work. In fact, according to the authors (Hu et al., unpublished data) the destruxins production of *Aschersonia* spp was very little in fields so it did not affect lady beetle.

Of course, whitefly has other natural enemies such as parasitoids. Whether destruxins are toxic to them and can influence their occurrence, physiological stages and growth, it is another task to be accomplished.

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Conflicts of interest

None declared.

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