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The Combination of Destruxin A with Insecticide Chlorantraniliprole Increases Virulence Against *Plutella xylostella* L.

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Authors' Contribution
SR and ZH conceived and designed the study. ZH performed all the experiments and analyzed the data. ZH and SA wrote the article. SR helped in finalizing the manuscript.

Key words
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Resistant population
Synergistic effect

ABSTRACT

Diamondback moth, *Plutella xylostella*, is a major worldwide pest of cruciferous vegetables and has developed high levels of resistance against many pesticides. The current research presents the joint action of destruxin A and chlorantraniliprole against resistant strain of *Plutella xylostella* were bioassayed under laboratory and field cage conditions. An apparent increase in adjusted larval mortality in a dose-dependent manner was observed when destruxin A and chlorantraniliprole were applied individually. The level of synergism between destruxin A and chlorantriniliprole was affected by the concentration of each component in the mixtures. The synergistic effects were observed in treatments (80.0+3.0), (40.0+1.0), (40.0+3.0) and (80.0+1.0), respectively. The treatments (40.0+3.0) and (80.0+0.3) were determined as additive effects according to their cumulative adjusted mortalities, Me and Chi-square values. In field cage experiments synergistic action was observed for 80.0+3.0 treatment with its cumulative mortality values of 69.93 / 87.05 / 98.87% at 48, 72 and 96 h post-treatment while additive effects were observed for treatments (40.0+0.3), (40.0+1.0) and (80.0+0.3).

INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella*, is a major pest of cruciferous vegetables worldwide (Wu *et al*., 2010; Ali *et al*., 2011; Zalucki *et al*., 2012; Guo *et al*., 2013; Lin *et al*., 2013). *P. xylostella* larvae feed on various plants of crucifer's family, both in field (cabbage, broccoli and canola) and greenhouse crops (Zhang *et al*., 2012). Insecticides has long been used as a control measure against this pest, however this species has now developed resistance to many pesticides (Sun *et al*., 2012). Unfortunately, most of these insecticides are harmful to environment and multiple side effects on beneficial arthropods have been observed (Biondi *et al*., 2012; Lu *et al*., 2012). In China, *P. xylostella* populations have become high level resistant to conventional insecticides (Ali *et al*., 2009; Yi *et al*., 2012; Lin *et al*., 2013). In addition, *Bacillus thuringensis* resistant strains have also been detected in different regions of the world (Tabashnik, 1992; Ferré and Van Rie, 2002; Ali *et al*., 2009).

Chlorantraniliprole belongs to ryanodine receptor modulator class of insecticides. This insecticide is highly effective against different insect pests, including those which are resistant to other categories of conventional insecticides (Cordova *et al*., 2006; Guo *et al*., 2013). Upon ingestion, it activates the release of internal calcium, leading to reduced feeding, lethargy, muscle paralysis and ultimate death of insect (Lahm *et al*., 2005). These characteristics make this insecticide a promising tool for insecticide resistant management. Chlorantraniliprole was registered in China during 2008 and since then it has been used in several crops against larval instars of different lepidopteran pests such as *P. xylostella, Spodoptera exigua, Chilo supperssalis* and *Cnaphalocrosis medinalis*, although the concentrations of insecticides may vary on different crops (Han *et al*., 2012; Guo *et al*., 2013). Toxicity studies against field populations of diamondback moth revealed that majority of populations across the world were susceptible to chlorantraniliprole (Chen *et al*., 2010; Silva *et al*., 2012; Hu *et al*., 2014). However, due to indiscriminate use of this chemical the risk of resistance development are notably higher. It has recently been reported that the *P. xylostella* from southern China displays a high level of resistance to chlorantraniliprole, whereas the *P. xylostella* from central and northern China possess low and moderate levels of resistance to chlorantraniliprole (Lin *et al*., 2013; Hu *et al*., 2014).

Destruxins are mycotoxins extracted from *Metarhizium anisopliae*, having molecular structure of cyclic hexadepsipeptide typically composed of 5 amino acids and an α-hydroxyl acid (Pedras *et al*., 2002). Different analogs of destruxin, such as dtxA (destruxin A), dtxB and dtxE are known for their insecticidal

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activities (Amiri *et al.*, 1999). Destruxins are known to inhibit the insect immune system which separates them from other available insecticides and suggests the possibility of a new calss of insecticides (Vey *et al.*, 2002). However, destruxins have not been applied under field conditions. Therefore, exploring the interaction of destruxins with other chemical insecticides can improve our knowledge about the toxicity of destruxins.

The main objective of this study was to evaluate the compatibility of chlorantraniliprole with destruxins for the management of resistant *P. xylostella* population and to optimize integrated pest management programs involving the use of chlorantraniliprole and destruxins against this pest.

MATERIALS AND METHODS

Plutella xylostella *and Chemicals*

Sensitive strain of *P. xylostella* (DBM) was obtained from stock cultures on *Brassica campestris* L. (Brassicaceae) kept for 3 years in greenhouse of the Engineering Research Center of Biological Control, South China Agricultural University, Guangzhou, Guangdong province, China. Resistant strain of DBM were collected from a farm (Farm population) and near expressway greenbelt (Expressway population) and cultured in laboratory for 3 generations before experiment. Plants were grown in plastic pots (Ø 15 cm, diameter). Sufficient slow-release fertilizer (N:P:K = 13:7:15; Shenzhen Batian Ecotypic Engineering, Xili Shenzhen, China) was added as required to maintain normal plant growth. The newly molted second instars of *P. xylostella* were gently removed from host plant leaves using a fine camel-hair brush (No. 00) and put on pieces of fresh *B. campestris* leaf (100-150 cm^2) for bioassay.

Chlorantaniliprole(3-bromo-4′ -chloro-1-(3-chloro-2-pyridyl)-2′ -methyl-6′ -(methylcarbamoyl) pyrazole-5-carboxanilide) was obtained from Dupont, China. Destruxin A powder was purified and prepared from *M. anisopliae* by following Hu *et al.* (2006). The purity of Destruxin A was 90.5% as determined by HPLC.

Toxicity of destruxin-A to sensitive and resistant strains of P. xylostella

Insecticidal activities of destruxin-A was tested against the second instar larvae of *P. xylostella* by using non-choice leaf disc (1.8 cm diameter) method. Destruxin-A was dissolved in acetone to a concentration of 320 mg/L and four lower concentrations (160, 80, 40 and 20 mg/L) were prepared by serial dilutions using deionized water containing 0.05% Tween 80. Newly molted second instar larvae were selected and starved for 2 h. *B. campestris* leaves were washed with distilled water and leaf discs (Ø 2 cm) were cut after drying. The leaf discs having second instar larvae of treatment groups were immersed in the test solution for 10 s and left to dry at room temperature. The leaf discs of control groups were treated with 0.05% Tween 80 solutions with acetone at the same concentration as the treatment solutions. Two pieces of treated discs were placed in a petri dish (9 cm) having a piece of moistened cotton pad. One larva was added to each petri dish, and each treatment was having 30 larvae. Each treatment and control was repeated three times with a new batch of insects and new test solutions. Leaf disks without the test solution treated were replaced every day. All treatments and controls were assayed at one time, using randomized groups of insects from a single batch. The insects were placed in an air-conditioned room at 25±2ºC. *P. xylostella* mortality was recorded daily after treatment, respectively. Larvae were considered dead if they showed no response to physical stimulus (Touch) and body lost its normal color.

Toxicity of chlorantaniliprole to sensitive and resistant strain of P. xylostella

Insecticidal activity of chlorantaniliprole was tested against the second instar larvae of *P. xylostella* by using non-choice leaf disc (Ø 2 cm) method. Chlorantaniliprole was dissolved in acetone to a concentration of 12.5 mg/L and four lower concentrations (2.5, 0.5, 0.1, and 0.2 mg/L) were prepared by serial dilutions using deionized water containing 0.05% Tween 80. Bioassay of newly molted second instar larvae of *P. xylostella* were treated and operated using the method as described above. Larvae were considered dead if they showed no response to physical stimulus (Touch) and body's color turned into black.

Joint action of destruxin-A and chlorantaniliprole against resistant strain of P. xylostella *under laboratory conditions*

The activities of different destruxin-A and chlorantaniliprole mixtures were tested against the second instar larvae of *P. xylostella.* Basis of the resulted of experiment toxicity of chlorantaniliprole, farm population was used on. Two concentrations of destruxin-A (40.0, 80.0 mg/L) were used according to the result of experiment toxicity of destruxin. The different mixtures of destruxin-A and chlorantaniliprole were prepared by serial dilutions with 0.05% Tween-80, and bioassays were carried out, using the methods previously described above section. One larva was added to each petri dish, and each treatment had 30 larvae. The different mixtures of destruxin-A (mg/L) and chlorantaniliprole (mg/L) were as follows:

T1=40.0+0, T2=40.0+0.3, T3=40.0+1.0, T4=40.0+3.0, T5=80.0+0, T6=80.0+0.3, T7=80.0+1.0, T8=80.0+3.0, T9=0+0.3, T10=0+1.0 and T11=0+3.0.

Control of resistant strain of P. xylostella *with destruxin-A and chlorantaniliprole under field conditions*

The activities of the mixtures of destruxin-A and chlorantaniliprole were tested against the second instar larvae of *P. xylostella* under field conditions. The different mixtures of destruxin-A and chlorantaniliprole were prepared as described above section. Adult *P. xylostella* farm populations were removed after 6 h of egg-laying from the plants covered with plastic screen in the field. Only newly molted second instar larvae were kept on the leaves of *B. campestris* plants and all the other life stages of *P. xylostella* were removed from leaves before experiment, with 3 larvae for one leaf per plant. The different test solutions were sprayed with a 500 ml hand sprayer to the leaves having second instar larvae of treatment groups. Each plant with treated leaf was covered with plastic screen in the field. The leaves of control groups were treated with 0.05% Tween 80 solutions, having acetone at the same concentration as the treatment solutions. Each treatment had 30 larvae, and the entire experiment was conducted three times on different dates. To monitor insects and observe larval mortalities daily, the methods previously described under above section were followed. The same mixtures of destruxin-A (mg/L) and chlorantaniliprole (mg/L) were used as above section.

Statistical analysis

Mortality was scored every 24 h after treatment and mortalities of all treatments after an arcsine transformation were analyzed using one-way analysis of variance (ANOVA). Means were separated by Duncan's Multiple Range Test (DMRT) when F-value was significant. The cumulative adjusted mortality was calculated as the cumulative mortality in treatment minus the cumulative mortality in control divided by one minus the cumulative mortality in control. Chi-square test was used to determine the kind of interaction between destruxin-A and chlorantaniliprole (SAS institute, 2000). The data were control-corrected (Abbott, 1925) first, and converted into proportion (*i.e.*, 0-100% → 0-1). The mortality expected for no interaction (additive effect) was calculated as follows:

$$Me=Ma+Mb*(1-Ma)$$
$$X^2=[(Mab-Me)*100]*[(Mab-Me)*100]/(Me*100)$$

Where Me is the expected mortality for additive mortality; Ma, Mb and Mab are the observed mortalities for agents destruxin-A, chlorantaniliprole, and their combination, respectively. Then P-value were looked up in a chi-square table for df=1. If Mab significantly <Me, it meant antagonism; if Mab significantly >me, it meant synergism. Otherwise the mortality was additive.

RESULTS

LC_{50} of destruxin-A

The mean cumulative adjusted mortalities for the second instar larvae of three *P. xylostella* populations treated with five different concentrations of destruxin-A are showed in Figure 1. The control mortalities for three populations of sensitive, expressway and farm populations at 72h post treatment were 5.56, 5.56 and 4.44%, respectively. Based on the mortality data, the concentration mortality response regression analysis for destruxin-A was calculated by assaying five concentrations against *P. xylostella.* The LC_{50} and 95% fiducial limit values of destruxin-A against *P. xylostella* are shown in Table I. The result of bioassay showed that there were no differences of LC_{50} values among the three DBM populations after 72 h of treatment.

Table I.- Regression analysis of probit mortality and log-concentration data of bioassay with destruxin-A against *P. xylostella* (72h).

Population	LC_{50} (mg/L)	95% Fiducial limit	χ^2	Slope
Sensitive	87.81 ± 8.30a	72.96–105.69	5.42	1.4712
Expressway	96.51 ±9.51a	79.56–117.07	5.08	1.4287
Farm	107.55 ± 11.13a	87.81–131.72	4.73	1.4003
F, df, P	9.7, 2, 1.08			

Note: LC_{50} values (Means ± SE) in the same column followed by different letters are significantly different (DMRT, $P < 0.05$).

Table II.- Regression analysis of probit mortality and log-concentration data of bioassay with chlorantaniliprole against three populations of *P. xylostella* (72h).

Population	LC_{50} (mg/L)	95% Fiducial limit	χ^2	Slope
Sensitive	0.205 ± 0.04 c	0.14–0.30	3.86	0.7789
Expressway	5.66 ± 2.35 b	2.51–12.75	1.08	0.5045
Farm	10.22 ±4.86 a	4.02–25.96	0.76	0.5142
F, df, P	79.36, 2, 0.0001			

Note: LC_{50} values (Means ± SE) in the same column followed by different letters are significantly different (DMRT, $P < 0.05$).

LC_{50} of chlorantriniliprole

The mean cumulative adjusted mortalities of second instar larvae of three populations of *P. xylostella* treated with the five different concentrations of chlorantriniliprole are given in Figure 2. Control

mortalities for sensitive, expressway and farm populations at 72 h post-treatment were 5.56, 3.33 and 3.33%, respectively. The LC_{50} and 95% fiducial limit values of chlorantriniliprole against *P. xylostella* are shown in Table II. There were significant differences in LC_{50} values among the three populations after 72 h treatment. The population in farm was the highest resistant population among three populations, with the LC_{50} value of 10.22 mg/L, almost 50 times as that of the sensitive population. The LC_{50} value of expressway population was 27 times as that of sensitive population, with the LC_{50} value of 5.66 mg/L.

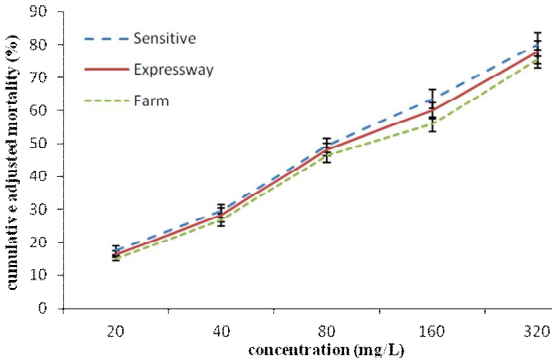

Fig. 1. Cumulative adjusted mortality of three populations of *P. xylostella* against destruxin-A (72 h).

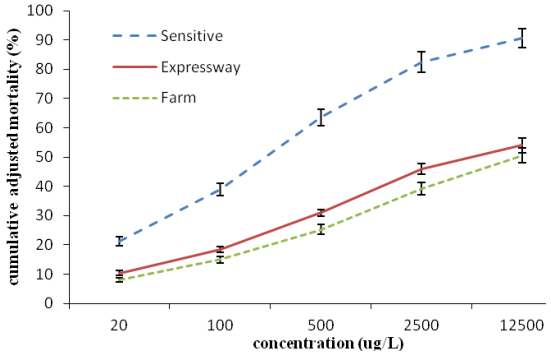

Fig. 2. The mean cumulative adjusted mortality of three populations of *P. xylostella* against chlorantaniliprole (72 h).

Effect of mixture of destruxin-A and chlorantaniliprole under laboratory condition

The mean cumulative adjusted morality of *P. xylostella* larvae, together with Me (expected mortality for additive mortality) and Chi-square values are presented in Table III. Most destruxin-A / chlorantriniliprole combinations tested caused high morality of DBM larvae and some of the combinations (marked with *) showed a substantial level of synergism. The percent cumulative mortalities in control were 2.22%, 2.22% and 3.33% for farm population, respectively, at 48, 72 and 96 h post-treatment. The level of synergism between destruxin-A and chlorantriniliprole was affected by the concentration of each component in the mixtures; *i.e*., the mortality of *P. xylostella* larvae increased with an increase in the concentration of destruxin-A and chlorantriniliprole and the mortality caused by cumulative effect lasted for an extended period (Table III). The cumulative adjusted larvae mortality in the treatments containing destruxin-A or chlorantriniliprole alone differed significantly from relevant mixtures of destruxin-A and chlorantriniliprole. The best synergistic effect was observed in treatment T8 (80.0+3.0) against farm population having cumulative mortality values of 83.11, 95.11 and 110.93 after 48h, 72h and 96 h treatment, respectively. Similar synergistic actions were also observed for treatments T3 (40.0+1.0), T4 (40.0+3.0) and T7 (80.0+1.0), respectively. The treatments T2 (40.0+0.3) and T6 (80.0+0.3) were determined as additive effects according to their cumulative adjusted mortalities, Me and Chi-square values (Table III).

Table III.- Mean percent cumulative adjusted mortality of *P. xylostella* on mixture of destruxin-A and chlorantraniliprole under laboratory condition.

Treatments	48 hrs	72 hrs	96 hrs
T1	18.30±0.92 e	26.46±0.98 f	35.23±1.01 g
T2	32.40±2.05 d (26.69, 1.22)	52.37±2.94 d (46.40, 0.77)	64.08±3.16 e (55.94, 1.19)
T3	47.20±1.91 c (39.92, 1.33)	75.03* ±3.75 b (53.60, 8.56)	87.92*±4.17 c (61.58, 11.27)
T4	68.99*±3.15 b (44.77, 13.10)	91.98*±5.63 a (57.31, 20.98)	103.94*±5.41b (66.58, 20.96)
T5	27.64 ± 1.04 d	40.68 ± 1.21 e	49.72 ± 1.46 f
T6	39.68±1.63 cd (35.07, 0.61)	66.42±2.41 c (56.76, 1.65)	74.03±2.98 d (65.80, 1.03)
T7	60.44*± 2.53 b (46.79, 3.99)	89.33* ±3.81 a (62.58, 11.45)	99.23* ±3.47b (70.17, 12.03)
T8	83.11*±2.74 a (51.08, 20.08)	95.11* ±4.28 a (65.56, 13.32)	110.93* ±3.76 a (74.06, 18.36)
T9	10.27±0.56 e	27.11±0.85 f	31.97±1.07 g
T10	26.46±0.87 de	36.91±1.42 e	40.68±1.24 fg
T11	32.40 ± 1.13 d	41.94 ± 1.39 e	48.40 ± 1.92 f
F, df, P	104.68, 10, 0.0001	129.43, 10, 0.0001	175.26, 10, 0.0001

Note: For treatment compositions see Materials and method Section 2.4. Means (M ± SE) in the same column followed by different letters are significantly different (DMRT, $P < 0.05$). Data on mean (±SE) mortalities were subjected to arcsine transformation prior to computation. Data in bracket shows Me (the expected mortality for additive mortality) subjected to arcsine transformation and the chi-square value, respectively. * represent the combined treatment having synergistic interaction through the data analysis.

Table IV.- Mean percent cumulative adjusted mortality of *P. xylostella* on mixture of destruxin-A and chlorantraniliprole in cages placed in the field.

Treatments	48 hrs	72 hrs	96 hrs
T1	13.84 ± 1.13 f	21.33 ± 1.27 f	28.62 ± 1.03 f
T2	24.38 ± 1.72 e	42.44 ± 3.04 d	50.33 ± 1.33 d
	(23.77, 0.02)	(39.06, 0.29)	(47.31, 0.19)
T3	36.44 ± 2.15 d	54.41 ± 3.25 c	67.32 ± 3.26 c
	(31.80, 0.68)	(45.78, 1.62)	(54.37, 3.09)
T4	57.07* ±4.32 b	73.50* ±3.11 b	83.48*±4.02 b
	(38.95, 8.43)	(50.70, 10.25)	(57.99, 11.20)
T5	18.50 ± 0.96 d	29.85 ± 0.93 ef	37.33 ±1.14 ef
T6	29.15 ±1.35 de	53.04 ± 1.35 c	62.89 ± 2.35 c
	(27.89, 0.06)	(45.66, 1.19)	(53.74, 1.56)
T7	46.49 ± 2.82 c	70.37* ±3.13 b	78.37*±2.76 b
	(35.49, 3.41)	(51.65, 6.78)	(59.94, 5.67)
T8	69.93 *±2.61 a	87.05 *±4.01 a	98.87 *±3.18 a
	(42.26, 18.13)	(56.03, 17.17)	(63.12, 20.24)
T9	11.53 ± 0.37 f	22.54 ± 0.74 f	26.18 ± 0.93 f
T10	20.84 ± 0.82 e	31.08 ± 1.07 e	36.07 ±1.03 ef
T11	29.15 ±1.02 de	37.33± 1.66 de	41.15 ± 1.32 e
F, df, P	97.51, 10, 0.0001	132.67, 10, 0.0001	173.19, 10, 0.0001

Note: For treatment compositions see Materials and method Section 2.4. Means (M±SE) in the same column followed by different letters are significantly different (DMRT, P<0.05). Data on mean (±SE) mortalities were subjected to arcsine transformation prior to computation. Data in bracket shows Me (the expected mortality for additive mortality) subjected to arcsine transformation and the chi-square value, respectively. * represent the combined treatment having synergistic interaction through the data analysis.

Effect of mixture of destruxin-A and chlorantaniliprole under field conditions

For the cage experiments in the field, cumulative adjusted mortality of *P. xylostella* farm population after the application of different concentration of destruxin-A and chlorantriniliprole showed different types of interaction effects (Table IV). The cumulative mortalities in the control were 3.33%, 5.56% and 5.56% for farm population at 48, 72 and 96 h post-treatment. The mortalities in treatments between destruxin-A and chlorantriniliprole for the combination of T8(8.0+3.0) / T7(80+ 1.0) / T4(40.0+3.0) at 48h, 72h and 96h, together with the Me and chi-square value obtained through the data analysis, showed a substantial synergism, whereas for all the other combinations an additive effects for the mortality was observed. The cumulative larval mortality for treatments containing only destruxin-A or chlorantriniliprole alone differed significantly from relevant mixtures of destruxin-A and chlorantriniliprole. The value of mortality revealed no significant difference between the treatments T7(80.0+1.0) and T4(40.0+3.0) at 72 and 96 h post-treatment. The best synergistic effect was observed in T8 (80.0+3.0) treatment with its cumulative mortality values of 69.93 / 87.05 / 98.87% at 48, 72 and 96 h post-treatment. The treatments of T2 (40.0+0.3), T3 (40.0+1.0) and T6 (80.0+0.3) showed additive effects according to their cumulative mortalities, Me and Chi-square values (Table IV).

DISCUSSION

Chlorantraniliprole is one of a new class of insecticides that is highly effective against *P. xylostella* L. (Chen *et al*., 2010). However, resistance to this insecticide in field populations in China presents a major risk to the effective life of this insecticide (Hu *et al*., 2012; Guo *et al*., 2013; Lin *et al*., 2013), *i.e*., high resistant population in field with LC_{50} up to 78.78 mg/L was reported in South China (Hu *et al*., 2012). Our results showed that DBM has now developed a high resistance to chlorantraniliprole in South China, with LC_{50} value of 5.66 and 10.22 mg/L, compared to sensitive population with LC_{50} value of 0.205mg/L.

Much information regarding to the toxicity of destruxin against different insect pests as well as its lethal and sublethal effects on different natural enemies were available (Thomsen and Eilenberg, 2000; Hu *et al*., 2007; Sree *et al*., 2008) but a few reports are available on compatibility of destruxin with other synthetic pesticides against resistant *P. xylostella* population. Therefore, current studies were conducted to observe the compatibility of destruxin A with chlorantraniliprole against *P. xylostella*, even the low dose of destruxin A could promote the effect of chlorantraniliprole against *P. xylostella.* During these studies, bioassay against *P. xylostella* by using different concentrations of destruxin A was carried out to determine its optimum concentration which was going to be used in remaining parts of these studies. Our results showed that resistant *P. xylostella* larvae of expressway / farm population were susceptible to destruxin A having LC_{50} value of 96.51 / 107.55 mg/L at 72 h post-treatment which is similar to the findings of previous studies (Yi *et al*., 2010, 2012).

Currently, the use of various insecticides in mixture have been used as an important tool for insect control, reduction in input costs and management of insect resistance against specific pesticides. The possible outcome of chemical interaction in mixture is influenced by different factors like target insect species, class of insecticide used, differences in biochemical properties of different insecticides and target protein of the chemicals (Rozman *et al*., 2001). Apart from this, selection of the interaction model in our study is also important because mortality of mixture comes from the combined action of toxin and insecticide. It is known that destruxin targets the insect immune system by damaging hemocytes, suppressing phagocytic activity and expression of various

microbial pepetides (Vey *et al*., 2002; Pal *et al*., 2007) whereas chlorantraniliprole binds to ryanodine receptors in muscles and nervous tissues resulting in an uncontrolled release of stored calcium from sarcoendoplasmic reticulum causing feeding cessation, lethargy, paralysis and ultimate death of target organism (Cordova *et al*., 2006; Guo *et al*., 2013). So, before assessing the combined toxicity of the mixture, bioassays were carried out to assess the effective concentration of destruxin A and chlorantraniliprole against *P. xylotella* for use in remaining parts of these studies.

Joint application of destruxin A and chlorantraniliprole resulted in different joint actions. There were additive and synergistic effects when different concentrations of destruxin A and chlorantraniliprole were mixed. The level of synergism was most evident under laboratory when 80.0 or 40.0 mg/L destruxin A was mixed with 1.0 or 3.0 mg/L chlorantraniliprole. This level of synergism is similar to the findings of Hu *et al*. (2010), who observed such synergism between destruxin and matrine. The possible reason behind the synergistic action of both chemicals can be related to their different mode and mechanism of target systems. Although, the mechanism of dtx was not very clearly illustrated yet to date, the theory of dtx as an inhibitor of insect immunity has been supporting by more researchers (Vilcinskas *et al*., 1997a,b; Vey *et al*., 2002). Inhibition of insect immunity associated the dtx treatment may have stressed the insect making them more susceptible to chlorantraniliprole reaching the target site. In addition slower killing speed of destruxin in combination with the quicker pest killing speed of chlorantraniliprole can also contribute to this synergistic action which is similar to the results of Yi *et al*. (2012).

Different combinations of destruxin A and chlorantraniliprole were applied to the cages in the field to further confirm the synergistic effect between them. The results of the cage experiments revealed the synergism between destruxin A and chlorantraniliprole for the combinations of 80.0 + 1.0/3.0 and 40.0 + 3.0, whereas for all the other combinations, an additive effect for the mortality was observed. The main reason of these differences can be the difference in environmental conditions, behavior of the pest to be controlled or application method of chemicals. Our results are similar to the other published studies on dual application of destruxin and other chemicals in which additive or synergistic mortality was observed (Hu *et al*., 2007; Yi *et al*., 2012).

These studies strongly recommend the joint application of destruxin A and chlorantraniliprole in field as well as greenhouses because of their synergistic action against *P. xylostella.* Such combination can also improve the infectious efficacy of destruxin and perhaps other fungal toxins. Current findings will also be of valuable use in integrated pest management programs which employ resistant insect species and fungal toxins concomitantly.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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