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A semi-field study to evaluate effects of sulfoxaflor on honey bee (*Apis mellifera*)

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Abstract

A semi-field study was conducted to investigate the effect of sulfoxaflor on honey bees and the residues in matrices after being used on cucumber. A suspension of 22% sulfoxaflor concentration was applied on cucumber at full bloom, at the rates of 75 g a.i./ha and 100 g a.i./ha, both with 2 applications at 6-day interval. Colonies were introduced into the tunnels one day after the first application, then mortality and behaviour of the test bees were observed and recorded every day. Cucumber flowers were taken and analysed for sulfoxaflor residues. The second application occurred 6 days later, then, observations of mortality and behaviour were conducted for 5 days, and then the colonies were removed from the tunnels and transported back to the apiary for 14 days additional observation. Besides, general conditions of the colonies were assessed before and after exposure, at the end of the field study. After being introduced into the tunnels, the colonies stabilized after 3 days' adaption. During the exposure period, sulfoxaflor showed lethal toxicity on bees, but the effect was less than that caused by dimethoate. After being removed from the tunnels, the mortalities of all groups fluctuated at a low level, indicating that sulfoxaflor had no long-term lethal effect on bees. During the whole exposure period, sulfoxaflor had no effect on the flight intensity of the bees. Meanwhile, sulfoxaflor had no obvious adverse effect on the strength and the condition of the test colonies. Residue analysis showed that: on the days of application, the residue of sulfoxaflor in cucumber flower was between 5.004 mg/kg and 5.832 mg/kg. After the applications, the residue of sulfoxaflor reduced gradually, until the 6th day after the first application, the residue reduced to 0.100~0.198 mg/kg, until the 5th day after the second application, the residue reduced to 0.155~0.304 mg/kg.

Key words: sulfoxaflor, negative side effects, honey bee, cucumber, semi-field trials.

Introduction

Sulfoxaflor is an insecticide that acts through a unique interaction with the nicotinic acetylcholine receptor in insects. It is an agonist of the nicotinic acetylcholine receptor (nAChR) and exhibits excitatory responses including tremors, followed by paralysis and mortality in target insects. While sulfoxaflor acts on the same receptor as the neonicotinoids, it is classified as its own subgroup (4C). The structure of sulfoxaflor makes it stable in the presence of a monooxygenase enzyme that was shown to degrade a variety of neonicotinoids in IRAC (Insecticide Resistance Action Committee) Group 4A, resulting in a lack of cross-resistance demonstrated in laboratory experiments (Sparks *et al.*, 2013).

Sulfoxaflor is classified as very highly toxic with acute oral and contact LD_{50} values of 0.05 and 0.13 µg a.i./bee, respectively, for adult honey bees (*Apis mellifera* L.). For larvae, a 7-d oral LD_{50} of >0.2 µg a.i./bee was determined (USEPA, 2010; 2013).

To investigate the effect of sulfoxaflor on honey bees under semi-field conditions, six tunnel studies have been conducted on cotton and other crops except for cucumber in US (USEPA, 2013). Sulfoxaflor is commonly applied on cucumber to control *Bemisia tabaci* (Gennadius) and cucumber could be visited by honey bee foragers. This study determined the effect of sulfoxaflor on honey bees and on the residues in matrices relevant to exposure of honey bee colonies following application of 22% sulfoxaflor suspension concentrate (SC) at full bloom. The results could add more evidence to the effect of sulfoxaflor on honey bees.

Materials and methods

Test chemicals

The test substance sulfoxaflor (22%w/W) SC was provided by Dow AgroSciences and the reference substance-dimethoate (40%w/W) EC (emulsifiable concentrate) was provided by the Institute for the Control of Agrochemicals, Ministry of Agriculture of the People's Republic of China.

Experimental field

The experimental field was located in Hengxi town, Jiangning District, Nanjing City, China. Not any crops had been planted in the field in the last two years. Before the start of the study, 12 tunnels were set up in the experimental field. The size of the tunnels was 48 m^2 (8 m long, 6 m wide). The maximum height of each tent was 3.5 m and the tent frames were covered with light nylon net with the mesh size of 2 mm (insect proof). 12 tunnels were randomly assigned to each application scenario, with three tunnels (replicates) for each application scenario.

Honey bee colonies

Honey bee (*Apis mellifera* L.) colonies were purchased from Nanjing Yuliang apiary. Twelve healthy and queen-right colonies with at least 10000 adult bees, 3 full combs with all brood stage each were used. The colonies were from one breeding line (sister queens newly cultivated in this year) in order to guarantee uniform bee material in all treatments.

No treatment against *Varroa* mites was performed at

least 4 weeks before start of the study. Furthermore, the following criteria for the nuclei were guaranteed: (a) at least 2 brood combs with all brood stages; (b) at least 1 honey and pollen combs; (c) bees are free of symptoms of nosema and other bee diseases. Hives used in the study were selected without conscious bias but any that were obviously unhealthy or damaged were not used.

Crop

Cucumber (*Cucumis sativus* L.) seeds (Jinchun 4#) were provided by Nanjing Institute of vegetables and flowers. Cucumber was planted two months before the test, and was managed according to the good agriculture practice (GAP). In order to control the downy mildew, 44% Metalaxyl-M chlorothalonil SC was used for three times (9th, 18th and 25th of June) prior to the first application of the test chemicals.

Experiment procedure

Treatments

The treatments were carried out at full bloom, in the early morning, when cucumber flowers were open. At the time of applications, the environmental conditions were recorded. The application scenarios were as the following:

- Application rate: 75 g sulfoxaflor a.i./ha, with 2 applications at 6-day interval (T1);
- Application rate: 100 g sulfoxaflor a.i./ha, with 2 applications at 6-day interval (T2);
- Negative control (tap water): applied on the same days as sulfoxaflor;
- Positive control (40% dimethoate EC, 100 g product/mu): in order to validate the test system and the exposure process, dimethoate was selected to be the reference substance and was applied on the same day with the second application of sulfoxaflor.

Sulfoxaflor, the reference substance and the tap water in negative control were all applied through spraying.

Introduction, keep and removal of the colonies

The colonies were introduced into the tunnels one day after the first application in the evening (one colony per tunnel). Each tunnel had one entrance, and the colonies were placed at the side opposite to the entrance and in direction to the tunnel. On the day of the second application, the entrances of the test hives were closed in the early morning (prior to the bee flight) and the hives were covered with a plastic sheet, before treatments. After application of treatments, the entrances were opened and the bees were released from the hives. The colonies were left in the tunnels for 11 days. One open container with water was placed into each tunnel. The surface of the water was covered with grasses to prevent the bees from drowning. Before introduction to the tunnels, the hives were maintained in the apiary (Huangmei town, Jurong city, Zhenjiang city, Jaingsu province, about 60 km away from the field site). After the exposure phase of the bees (five days after the second application), the hives were transported back to the same apiary for continuing feeding and observation.

Assessments of the toxicity endpoints

Behaviour of honey bees:

In order to collect and record the dead bees in and outside the hive, a bee trap was set around the hive entrance. And the paths in the middle of the tunnel and around the tunnel were covered with blue nylon nets. The number of dead bees in the bee trap and on the blue nylon nets was recorded every day. On the day of application, the recording frequency increased, such as 2 h, 4 h and 6 h after application and in the nightfall when bees stop flying. Besides, aggressive behaviour and other toxic symptoms were recorded daily.

Flight intensity:

Bee flight intensities were observed in the morning, noon and evening from the day after bee introduction to the day before the second application, 5 days after the second application. And on the day of application, the recording frequency increased, such as 2 h, 4 h and 6 h after application. At each assessment time the number of bees that are both foraging on flowering cucumbers and flying over the crop were counted on a square of 1 m^2 for 1 minute. In each assessment time and plot, the square (3 sites) observed was chosen randomly.

Colonies conditions and sizes:

The conditions and sizes of the colonies were assessed for three times: before and after exposure, at the end of the field study.

The following parameters were visually estimated and recorded when assessing the colonies conditions: presence of the queen (healthy, presence of eggs, presence of queen cells); visual assessment of the pollen and nectar storage area; visual assessment of the area containing eggs, larvae and capped cells. For the estimation of colony conditions, each side of a comb was divided into 32 equally sized areas. The number of areas per comb side fully and/or half covered with eggs, larvae, capped cells, pollen, nectar and adult bees, were estimated and recorded. For each comb side, the number of areas was summed up to a maximum of the equally sized area number. This was carried out for all combs of each hive. Afterwards the mean values were calculated for each hive.

The size of each colony was measured by weighing. When bees were present on the walls inside the bee hives, they were estimated accordingly and were added to the number of areas of one or more of the comb sides.

Flower collections

Cucumber flowers were taken and analysed for sulfoxaflor. Sampling started from the beginning of the test till the end of exposure. Every day, 10 flowers (about 2 g) for each tunnel were collected. All samples were timely transported to the analysis site under low temperature and were subsequently stored deep frozen at about −18 °C.

LC-MS/MS analysis of flowers

Weigh 2 g sample of cucumber flower and put the sample into 80 mL centrifuge tube, add 10.0 mL ace-

tonitrile, 1 g anhydrous magnesium sulphate and 1 g sodium chloride successively and mix them, use homogenizer to homogenize and extract them for 2 minutes with the speed of 15000 rpm, use supercentrifuge to centrifuge for 5 minutes with the speed of 8000 rpm, get 2 mL supernatant and put the supernatant into 10mL centrifuge tube, and add 0.05 g PSA, 0.01 g active carbon and 0.15 g anhydrous magnesium sulphate to purify, swirl them for 3 minutes and centrifuge them for 5 minutes with the speed of 8000 rpm, get supernatant to filter through 0.22 μm filter membrane and wait to be measured by LC-MS/MS.

Statistical analysis

All data were expressed as means ± SD. SPSS 22.0 was used to perform statistical analysis. The relevant values were analysed through One-way Analysis of Variance (ANOVA) followed by LSD test. Statistical significance was considered at $P < 0.05$ and highly significant difference was considered at $P < 0.01$.

Results

Behaviour of the bees

During the exposure period, many bees were clustering at the top of the tunnel, and it is bees' native response when they were confined within the tunnels. Except that, not any other abnormal activities were observed in the sulfoxaflor treatment groups and the blank control groups. But bees in the positive control groups were fairly irritable after the application of reference substance (dimethoate).

Mortalities

After being introduced into the tunnels, a small number of bees died. One day after the introduction (1DAE), large number of bees died in each group for the confinement of the tunnels. The number of dead bees of T1 group was the highest, which was extremely significantly different with that of the blank control ($P < 0.01$). And the number of dead bees of untreated positive control group was lower than that of T1 group, but was also extremely significantly different with that of the blank control ($P < 0.01$). The number of dead bees of T2 group was lower than that of the untreated positive control group but apparently higher than that of the blank control ($P > 0.05$). It could be supposed that the bee colonies were confined in the tunnels. Two days after the introduction (2DAE), the numbers of dead bees of blank control group and T2 group increased, while the number of dead bees of T1 and the untreated positive control decreased. The numbers of dead bees of T1 group and T2 group were still higher than that of the blank control, but the differences were not significant ($P > 0.05$). Three days after the introduction (3DAE), the numbers of dead bees of all groups all decreased, the numbers of dead bees of T1 group and T2 group were obviously higher than that of the blank control, and the differences were extremely significant ($P < 0.01$). Four days after the introduction (4DAE), the numbers of dead bees of all groups continued decreasing, the numbers of dead bees of T1 group and T2 group were still higher than that of the blank control, and the difference between T2 group and blank control was significant ($P < 0.05$). Five days after the introduction (5DAE), the numbers of dead bees of all groups continued decreasing, the numbers of dead bees of T1 group and T2 group were still higher than that of the blank control, but the differences were not significant ($P > 0.05$). Six days after the introduction (6DAE), the second application was conducted; meanwhile, the positive control substance (dimethoate) was applied. After the application, the numbers of dead bees of all groups increased significantly, among which the number of dead bees of positive control group was the highest, the number of dead bees of T1 group was lower than that of the positive control group but much higher than that of the T2 group. The numbers of dead bees of positive control group, T1 group and T2 group were extremely significantly higher than that of blank control group ($P < 0.01$). When comparing with the positive control group, the difference between the T1 group and the positive control group was not significant ($P > 0.05$), but the difference between the T2 group and the positive control group was significant ($P < 0.05$) with the number of dead bees of T2 group significantly lower than that of the positive control group. One day after the second application (7DAE), the numbers of dead bees of all groups all decreased, but the number of dead bees of positive control group was still the highest, the number of dead bees of T2 group was lower than that of the positive control group but much higher than that of the T1 group. The differences between the positive control group, the T2 group and the blank control group were extremely significant ($P < 0.01$), and the differences between the T1 group and the blank control group were significant ($P < 0.05$). The numbers of dead bees of T1 group and T2 group were significantly lower than that of the positive control group ($P < 0.05$). Two days after the second application (8DAE), the numbers of dead bees of all groups continued decreasing, the number of dead bees of positive control group was still the highest, and the number of dead bees of T1 group was higher than that of the T2 group. The difference between the positive control group and the blank control group was extremely significant ($P < 0.01$). The difference between the T1 group and the blank control group was significant ($P < 0.05$). The difference between the T2 group and the blank control group was not significant ($P > 0.05$). The number of dead bees of T2 group was significantly lower than that of the positive control group ($P < 0.05$). Three days after the second application (9DAE), the numbers of dead bees of all groups continued decreasing, the number of dead bees of positive control group was still the highest, and the number of dead bees of T1 group was still higher than that of the T2 group. The difference between the positive control group and the blank control group was extremely significant ($P < 0.01$). The difference between the T1 group and the blank control group was significant ($P < 0.05$). The difference between the T2 group and the blank control group was not significant ($P > 0.05$). The numbers of dead bees of T1 group and T2 group were significantly

lower than that of the positive control group ($P < 0.05$). Four days after the second application (10DAE), the numbers of dead bees of T2 group and blank control group continued decreasing, while the numbers of dead bees of T1 group and positive control group increased, among which the number of dead bees of positive control group was still the highest, the number of dead bees of T1 group was much higher than that of T2 group. The differences between the positive control group, the T1 group and the blank control group were extremely significant ($P < 0.01$). The difference between the T2 group and the blank control group was not significant ($P > 0.05$). The differences between the T1 group, T2 group and the positive control group were extremely significant ($P < 0.01$). Five days after the second application (11DAE), the numbers of dead bees of all groups all decreased, among which the number of dead bees of positive control group was still the highest, the number of dead bees of T1 group was much higher than that of T2 group. The numbers of dead bees of T1 group was significantly lower than the positive control group ($P < 0.05$). The difference between the T2 group and the positive control group was extremely significant ($P < 0.01$). After being removed from the tunnels and till the end of the experiment, the numbers of dead bees of all groups fluctuated at a low level (figure 1).

Two hours after application, large number of bees died in T1 group, T2 group and positive control group, and the numbers of dead bees in these groups were higher than that of the blank control group, the differences were extremely significant ($P < 0.01$). Four hours after application, the numbers of dead bees of all groups increased, among which the number of dead bees of positive control group was the highest, and the number of dead bees of T1 group was higher than that of T2 group. But the numbers of dead bees in these groups were all higher than that of the blank control group. The differences between the positive control group, the T1 group and the blank control group were extremely significant ($P < 0.01$). The difference between the T2 group and the blank control group were significant ($P < 0.05$). Six hours after application, the numbers of dead bees in T1 group, T2 group and positive control group decreased. The number of dead bees of positive control group was significantly higher than that of the blank control group ($P < 0.05$). The differences between T1 group, T2 group and blank control group were not significant ($P > 0.05$). Eight hours after application, the numbers of dead bees in all groups continued decreasing, to a consistent level (figure 2).

Of all the groups, the total amount of dead bees of positive control group was the highest, and the total amount of dead bees of T1 group was lower than that of the positive control group but higher than that of the T2 group. The total amount of dead bees of blank control group was the lowest. The differences between positive control group, T1 group and blank control group were extremely significant ($P < 0.01$). The difference between T2 group and blank control group was significant ($P < 0.05$). The total amount of dead bees of T2 group was significantly lower than that of the positive control group ($P < 0.05$) (figure 3).

From the above, the following could be concluded: after being introduced into the tunnels, the colonies stabilized after 3 days' adaption. During the exposure period, the mortalities of both treatment groups were higher than that of the blank control group, but lower than that of the positive group, which indicated that sulfoxaflor had some influence on bees, but the effect was less than that caused by dimethoate, the positive substance. The acute effects of sulfoxaflor on bees appeared within 2 h after the second application. After being removed from

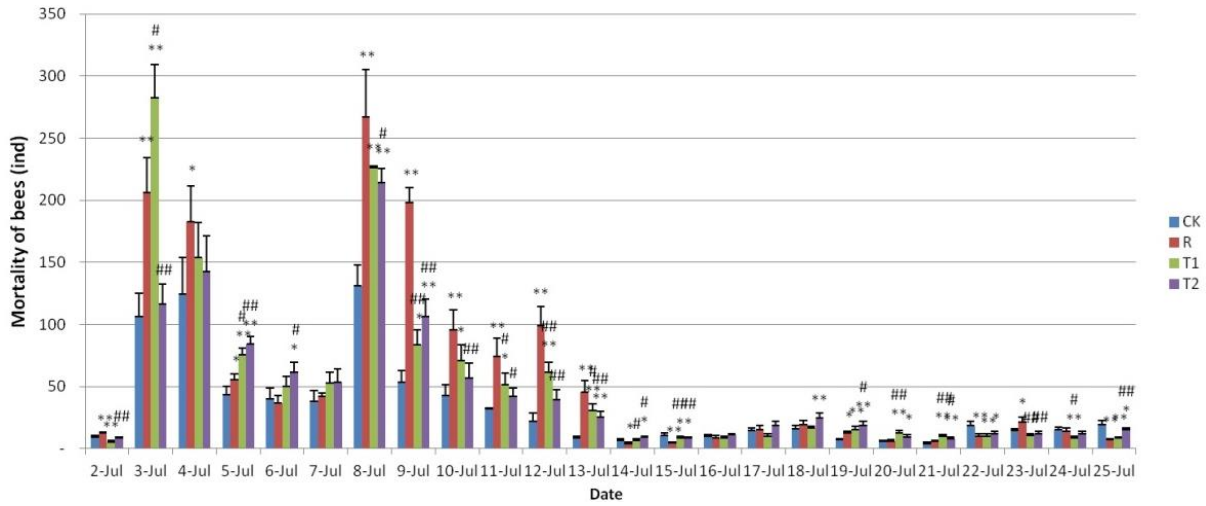

Figure 1. Change of individual (ind) bee mortality in each group with time from the beginning of exposure to the end of the test. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July 1 and July 8. (*) = significantly different between blank control and positive control group or treatment groups, $P < 0.05$; (**) = highly significantly different between blank control and positive control group or treatment groups, $P < 0.01$; (#) = significantly different between positive control group and treatment groups, $P < 0.05$; (##) = highly significantly different between positive control group and treatment groups, $P < 0.01$.

the tunnels, the mortalities of all groups fluctuated at a low level, indicating that sulfoxaflor had no long-term lethal effect on bees.

Flight intensities

During the whole exposure period, the bee flight intensities of all groups at the same time of the same day were not significantly different. When compared among different time periods, the bee flight intensities at 7:00 and 11:00 were higher than that at 16:00 (figure 4).

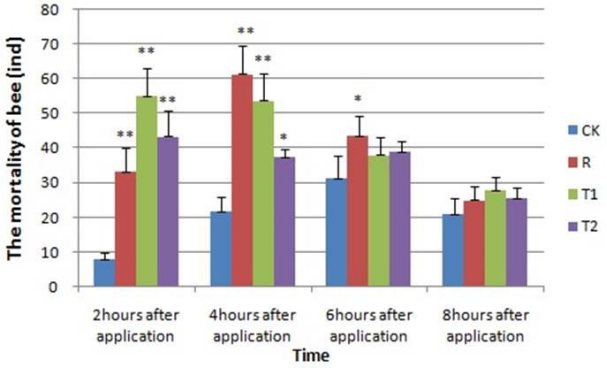

Figure 2. Bee mortality of each group on the day of second application (July 8). CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July 1 and July 8. (*) = significantly different between blank control and positive control group or treatment groups, $P < 0.05$; (**) = highly significantly different between blank control and positive control group or treatment groups, $P < 0.01$.

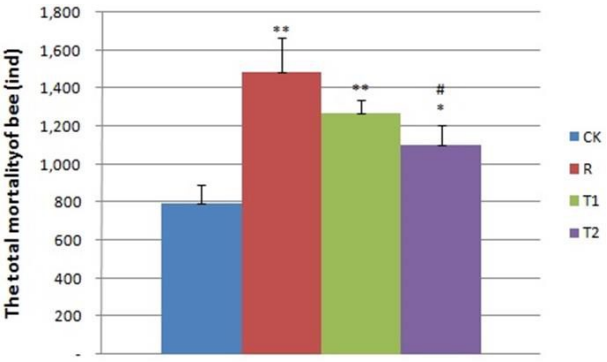

Figure 3. Total amount of dead bees in each group during the whole experiment period. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July 1 and July 8. (*) = significantly different between blank control and positive control group or treatment groups, $P < 0.05$; (**) = highly significantly different between blank control and positive control group or treatment groups, $P < 0.01$; (#) = significantly different between positive control group and treatment groups, $P < 0.05$.

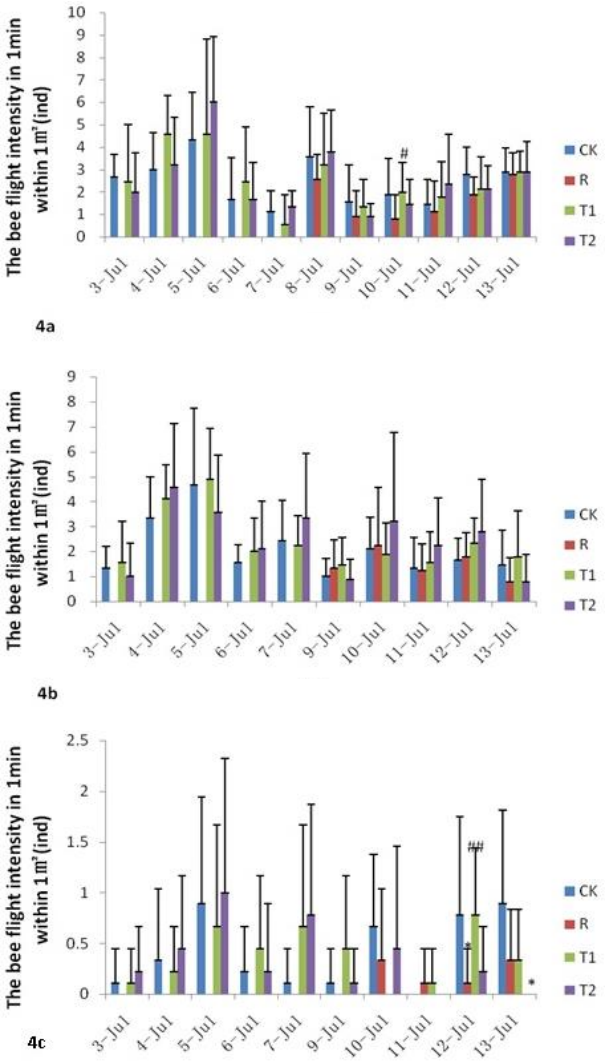

Figure 4. Change of bee flight intensity of each group from the beginning to the end of exposure. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July 1 and July 8. **4a:** bee flight intensity at 7:00; **4b:** bee flight intensity at 11:00; **4c:** bee flight intensity at 16:00. (#) = significantly different between positive control group and treatment groups, $P < 0.05$; (##) = highly significantly different between positive control group and treatment groups, $P < 0.01$.

Colonies conditions and sizes

Before exposure, there were no significant differences in the total amount of bees between groups ($P > 0.05$). On the second day of the end of exposure, the total amount of bees of positive control group became the highest, and the total amount of bees of T1 group was lower than that of the positive control group but higher than that of the blank control group. The total amount of bees of T2 group was the lowest, significant lower than the positive control group ($P < 0.05$). On 14 days after the end of exposure, the differences in the total amount of bees between groups were not significant ($P > 0.05$) (figure 5).

The total amounts of bees in each group on the second day of the end of exposure and on 14 days after the end of exposure were not significantly different with that of the first assessment ($P > 0.05$) (figure 6).

Before exposure, there was pollen stored in each hive, and the colonies were healthy with different portions of different brood stages, including pupae, larval, young bees. At the end of exposure, the pollen storage in each hive decreased to zero while the proportion of bees at each developmental stage did not change obviously. On 14 days after the end of exposure, there appeared pollen in each hive, also with different portions of different brood stages (figure 7).

Colony condition assessment showed that: the pollen storage during the exposure period decreased to zero, while other endpoints such as the colony strength, the proportion of bees at each developmental stage did not change obviously. Under this experiment condition, sulfoxaflor had no obvious adverse effect on the strength and the condition of the test bees.

Sulfoxaflor residues on cucumber flowers

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of sulfoxaflor in cucumber flower were calculated according to 0.01 mg/kg added recovery experiment. LOD was three-time standard deviation (0.0018 mg/kg) while LOQ was ten-times standard deviations (0.006 mg/kg).

The limit of detection (LOD) and limit of quantitation (LOQ) of dimethoate in cucumber flower were calculated according to 0.02 mg/kg added recovery experiment. LOD was three-time standard deviation (0.0009 mg/kg) while LOQ was ten-times standard deviations (0.003 mg/kg).

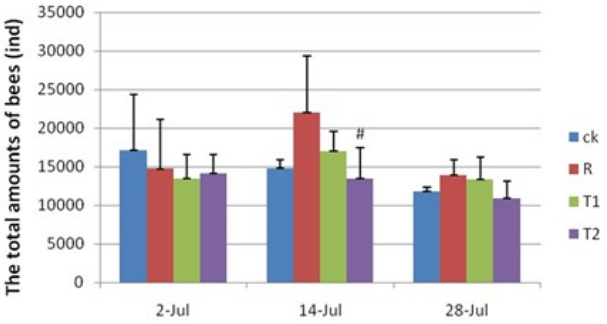

Figure 5. Comparison of the total amounts of bees at the same time. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July 1 and July 8.

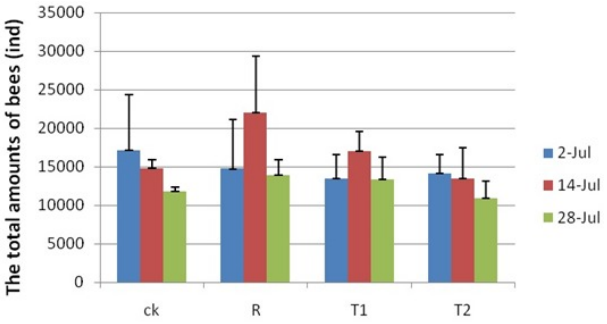

Figure 6. Comparison of the total amounts of bees in same group before exposure, on the second day of the end of exposure and on 14 days after the end of exposure. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July 1 and July 8.

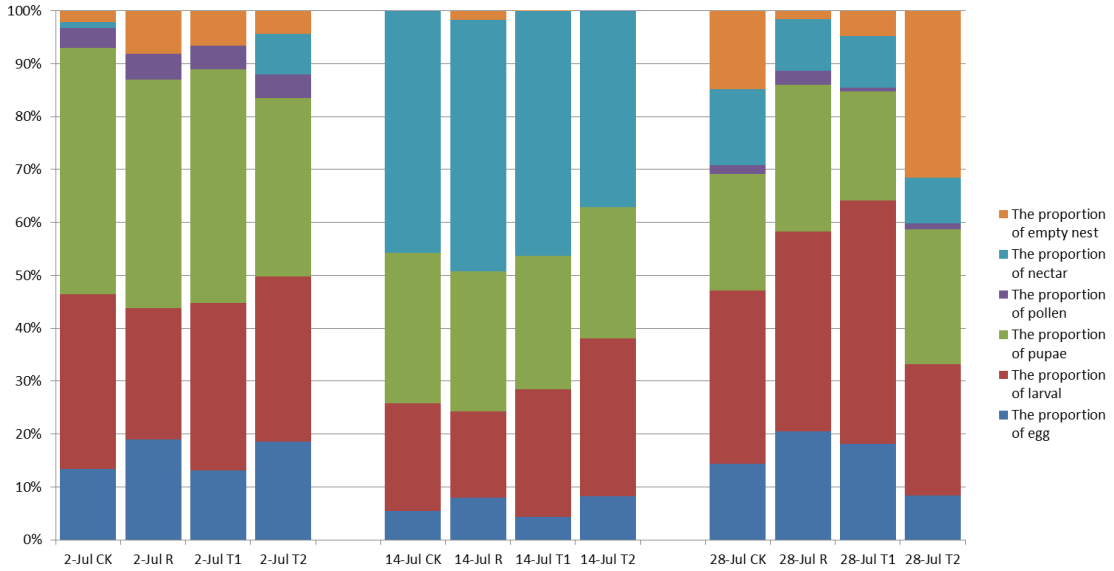

Figure 7. Change of colony conditions before exposure, on the second day of the end of exposure and on 14 days after the end of exposure. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha on July 1 and July 8.

Recovery rate

The average added recovery rates of three concentrations (0.01 mg/kg, 0.1 mg/kg, and 1 mg/kg) of sulfoxaflor in cucumber flower were respectively 88.18%, 90.10% and 93.99%. Mutation coefficients (relative standard deviations) were respectively 7.5%, 5.2% and 4.7%.

The average added recovery rates of three concentrations (0.02 mg/kg, 0.1 mg/kg, and 1 mg/kg) of dimethoate in cucumber flower were respectively 74.66%, 81.83% and 71.28%. Mutation coefficients (relative standard deviations) were respectively 9.4%, 6.6% and 5.9%.

Summary of analytical results

Residue determination results of sulfoxaflor and dimethoate could be seen in tables 1-3.

Table 1. Relative average residues of sulfoxaflor in cucumber flower after the first application (mg/kg).

Date	CK	First application T1 (mg/kg)		T2 (mg/kg)	
		Residue	Mean Residue	Residue	Mean Residue
July 1	<0.006	5.288	5.004 ± 1.206	6.576	5.283 ± 1.424
	<0.006	6.043		3.756	
	<0.006	3.681		5.519	
July 2	<0.006	0.448	0.667 ± 0.207	1.553	1.356 ± 0.217
	<0.006	0.858		1.123	
	<0.006	0.696		1.394	
July 3	<0.006	0.475	0.601 ± 0.267	0.731	1.085 ± 0.323
	<0.006	0.420		1.162	
	<0.006	0.908		1.362	
July 4	<0.006	0.410	0.653 ± 0.435	0.480	0.457 ± 0.118
	<0.006	1.155		0.329	
	<0.006	0.394		0.562	
July 5	<0.006	0.249	0.307 ± 0.051	0.383	0.332 ± 0.107
	<0.006	0.345		0.209	
	<0.006	0.328		0.403	
July 6	<0.006	0.030	0.144 ± 0.108	0.140	0.189 ± 0.091
	<0.006	0.245		0.295	
	<0.006	0.157		0.133	
July 7	<0.006	0.039	0.100 ± 0.077	0.254	0.198 ± 0.048
	<0.006	0.074		0.169	
	<0.006	0.187		0.171	
Half-life period (days)		1.25		1.30	

Table 2. Relative average residues of sulfoxaflor in cucumber flower after the second application (mg/kg).

Date	CK	Second application T1 (mg/kg)		T2 (mg/kg)	
		Residue	Mean Residue	Residue	Mean Residue
July 8	<0.006	5.584	5.510 ± 0.101	6.625	5.832 ± 0.728
	<0.006	5.551		5.676	
	<0.006	5.395		5.195	
July 9	<0.006	1.863	1.647 ± 0.302	4.297	3.433 ± 1.086
	<0.006	1.776		3.787	
	<0.006	1.302		2.214	
July 10	<0.006	0.787	0.548 ± 0.243	1.238	1.109 ± 0.267
	<0.006	0.555		0.802	
	<0.006	0.302		1.287	
July 11	<0.006	0.394	0.572 ± 0.235	1.097	0.891 ± 0.199
	<0.006	0.838		0.877	
	<0.006	0.483		0.700	
July 12	<0.006	0.137	0.228 ± 0.106	0.421	0.465 ± 0.143
	<0.006	0.344		0.349	
	<0.006	0.204		0.625	
July 13	<0.006	0.130	0.155 ± 0.034	0.323	0.304 ± 0.026
	<0.006	0.179		0.314	
	<0.006	<0.006		0.274	
Half-life period (days)		1.02		1.15	

Table 3. Relative average residue of dimethoate in cucumber flower (Unit: mg/kg).

Date	R (mg/kg)	
	Residue	Mean residue
July 8	15.388 13.650 14.250	14.429 ± 0.883
July 9	3.110 7.715 2.065	4.297 ± 3.006
July 10	0.630 0.905 0.385	0.640 ± 0.260
July 11	1.130 0.660 0.470	0.753 ± 0.340
July 12	0.045 < 0.003 < 0.003	0.045 ± 0
July 13	< 0.003 < 0.003 < 0.003	<0.003
Half-life period (days)	0.52	

Residue analysis showed that: the day on the first application, the residue of sulfoxaflor in cucumber flower was between 5.004~5.283 mg/kg. Between the first and the second application, the residue of sulfoxaflor reduced gradually, until the 6th day after the first application, the residue reduced to 0.100~0.198 mg/kg. After the second application, the residue increased evidently, with the rate of 5.510~5.832 mg/kg. After that, the residue reduced, until the 5th day after the second application, the residue reduced to 0.155~0.304 mg/kg.

At 0 day, the concentration of dimethoate was 14.429 mg/kg. Then the residue amount decreased and the half-life period was 0.52 day, on the sixth day after the application the residue amount was below LOQ (0.003 mg/kg).

Discussion

The semi-field test revealed that: after being introduced into the tunnels, the colonies experienced a three-day adaption. And during the exposure period, the mortalities of both treatment groups (75 g a.i./ha and 100 g a.i./ha) were higher than that of the blank control group, but lower than that of the positive group, which indicated that sulfoxaflor had acute toxicity on bees, but the effect was less than that caused by the positive substance (dimethoate). After being removed from the tunnels, the mortalities of all groups were fluctuated at a low level, indicating that sulfoxaflor had no sub-lethal effect on bees. During the whole exposure period, the bee flight intensities of all groups were not significantly different, indicating that sulfoxaflor had no effect on the flight intensity of the bees. The pollen storage during the exposure period decreased to zero, while other endpoints such as the colony strength, the proportion of bees at each developmental stage did not change. Under this experiment condition, sulfoxaflor had no observed adverse effect on the strength and the condition of the test bees.

The residue of sulfoxaflor in cucumber flower was between 5.004~5.283 mg/kg on the day of first application. Then reduced gradually to 0.100~0.198 mg/kg until the day of second application. After the second application, the residue increased evidently, with the rate of 5.510~5.832 mg/kg. After that, the residue reduced to 0.155~0.304 mg/kg at the 5th day after the second application. According to Rortais *et al*. (2005), the maximum food ingestion of bee is 0.128 g per bee, in our study, the maximum exposure rate in flower is 5.832 mg/kg, then the maximum exposure dose could be the product of the maximum exposure rate and the maximum food ingestion of bee, that is 0.746 μg a.i./bee, significantly higher than the acute oral and contact LD50 values of sulfoxaflor (0.05 and 0.13 μg a.i./bee, respectively) (USEPA, 2010; 2013), which can explain the acute mortality of the bees in treatment groups.

Six tunnel studies conducted on cotton and other crops except for cucumber in US revealed that at the application rates used, the direct effects of sulfoxaflor on adult forager bee mortality and the occurrence of behavioural abnormalities is relatively short lived, lasting 3 days or less. In contrast, the reference toxicant used in these studies indicated much greater, sustained mortality over the duration bees were housed in the tunnels (USEPA, 2013). The conclusions of all these studies were in accordance with our study. The results of our study could add more evidence to the effect of sulfoxaflor on honey bees. Although we could not find significant long term lethal effect of sulfoxaflor on bees under the semi-field test conditions, long term exclusive ingestion of the maximal residue levels of sulfoxaflor (3 ppm a.i.) may induce substantial bee mortality (Zhu *et al*., 2017a; 2017b). Meanwhile, the toxicity to bees could be synergized and effects such as significant synergistic mortality could be observed when mixing with other pesticides, many other chemicals and factors (Zhu *et al*., 2017a; 2017b; Sgolastra *et al*., 2017; Chauzat *et al.*, 2006*;* Tosi *et al.*, 2017). Therefore, further studies should be conducted on the long term effect of sulfoxaflor on bees and of course measures to reduce the acute risk of sulfoxaflor on bees are needed.

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