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.

Acknowledgements

The authors thank Dow AgroSciences, China for the funding of this study, besides, this research did not receive any other grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no conflict of interest.

References

CHAUZAT M., FAUCON J., MARTEL A., LACHAIZE J., COUGOULE N., AUBERT M., 2006.- A survey of pesticide residues in pollen loads collected by honey bees in France.- *Journal of Economic Entomology*, 99: 253-262.