

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

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

- EPPO, 2010.- Efficacy evaluation of plant protection products: side-effects on honeybees.- *EPPO Bulletin*, 40: 313-319.
- RORTAIS A., ARNOLD G., HALM M. P., TOUFFET-BRIENS F., 2005.- Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees.- *Apidologie*, 36: 71-83.
- SGOLASTRA F., MEDRZYCKI P., BORTOLOTTI L., RENZI M. T., TOSI S., BOGO G., TEPER D., PORRINI C., MOLOWNY-HORAS R., BOSCH J., 2017.- Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species.- *Pest Management Science*, 73: 1236-1243.
- SPARKS T. C., WATSON G. B., LOSO M. R., GENG C., BABCOCK J. M., THOMAS J. D., 2013.- Sulfoxaflor and the sulfoximine insecticides: chemistry, mode of action and basis for efficacy on resistant insects.- *Pesticide Biochemistry and Physiology*, 107: 1-7.
- TOSI S., NIEH J. C., SGOLASTRA F., CABBRI R., MEDRZYCKI P., 2017.- Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees.- *Proceedings of the Royal Society B, Biological science*, 284: 20171711.
- USEPA, 2010.- Response to public comments on EPA's "Proposed Registration of the New Active Ingredient Sulfoxaflor for Use on Multiple Commodities, Turf grass, and Ornamentals", EPA-HQ-OPP-2010-0889.
- USEPA, 2013.- Registration decision for the new active ingredient sulfoxaflor. "Registration of the New Active Ingredient Sulfoxaflor for Use on Multiple Commodities, Turf grass and Ornamentals", EPA-HQ-OPP-2010-0889-0396.
- ZHU Y. C., YAO J., ADAMCZYK J., LUTTRELL R., 2017a.- Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*).- *PLoS ONE*, 12 (6): e0178421.
- ZHU Y. C., YAO J., ADAMCZYK J., LUTTRELL R., 2017b.- Synergistic toxicity and physiological impact of imidacloprid alone and binary mixtures with seven representative pesticides on honey bee (*Apis mellifera*).- *PLoS ONE*, 12 (5): e0176837.

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