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

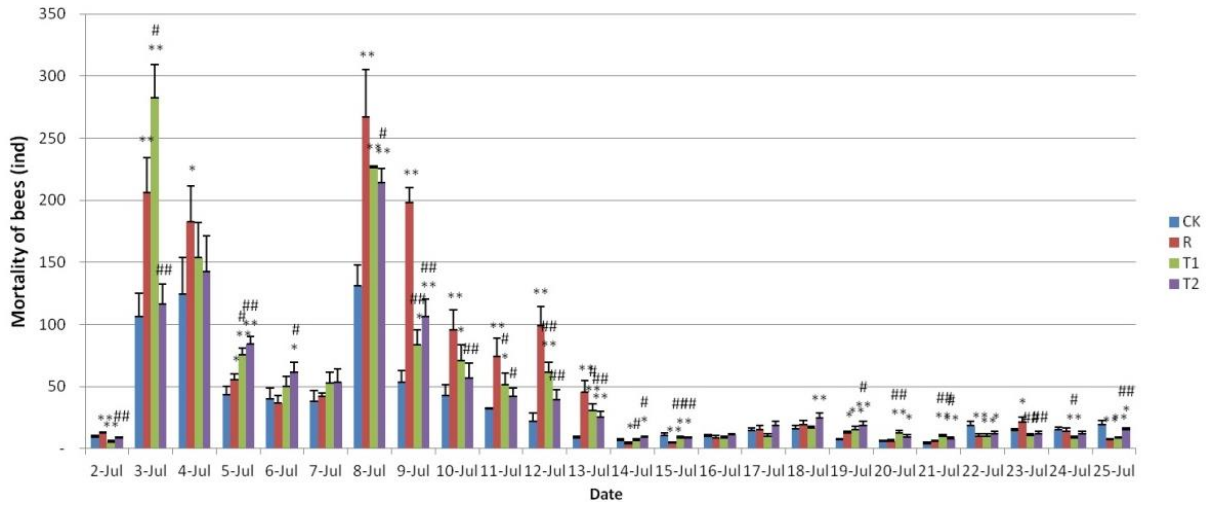

Figure 1. Change of individual (ind) bee mortality in each group with time from the beginning of exposure to the end of the test. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: 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).

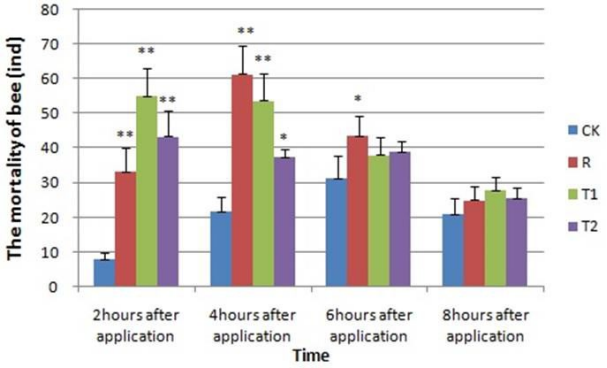

Figure 2. Bee mortality of each group on the day of second application (July 8). CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied on July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

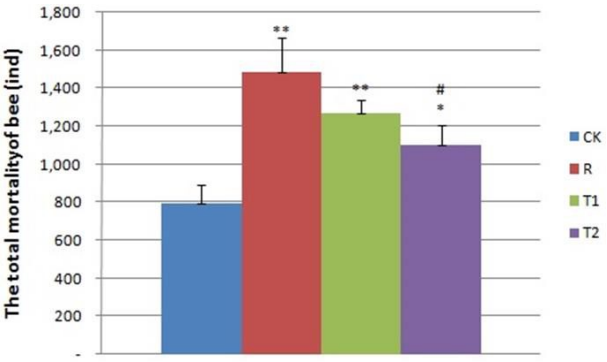

Figure 3. Total amount of dead bees in each group during the whole experiment period. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha applied 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, $P < 0.05$.

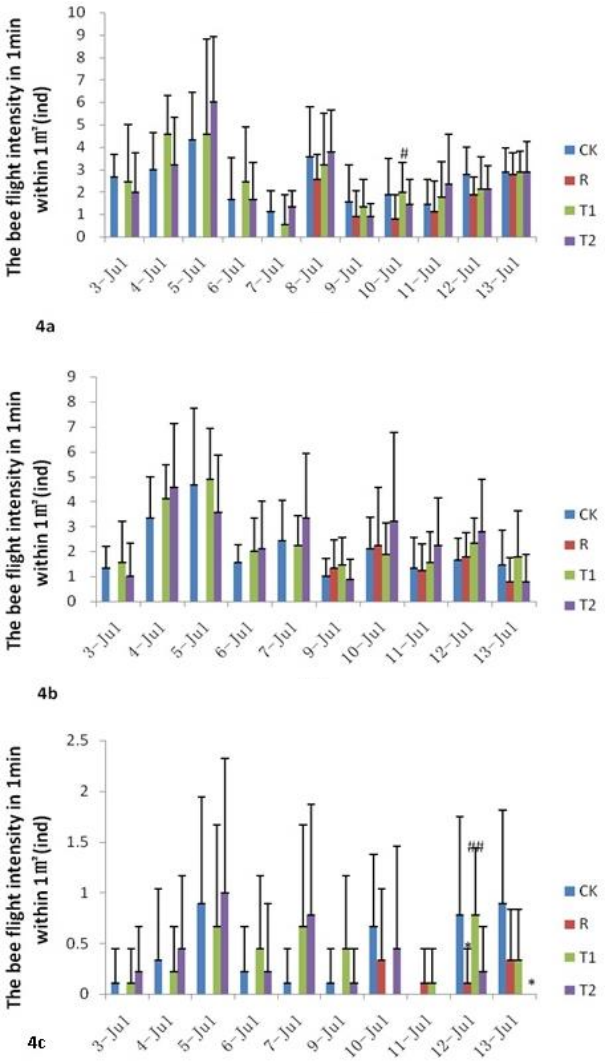

Figure 4. Change of bee flight intensity of each group from the beginning to the end of exposure. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8; T2: sulfoxaflor treatment, 100 g a.i./ha 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).