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

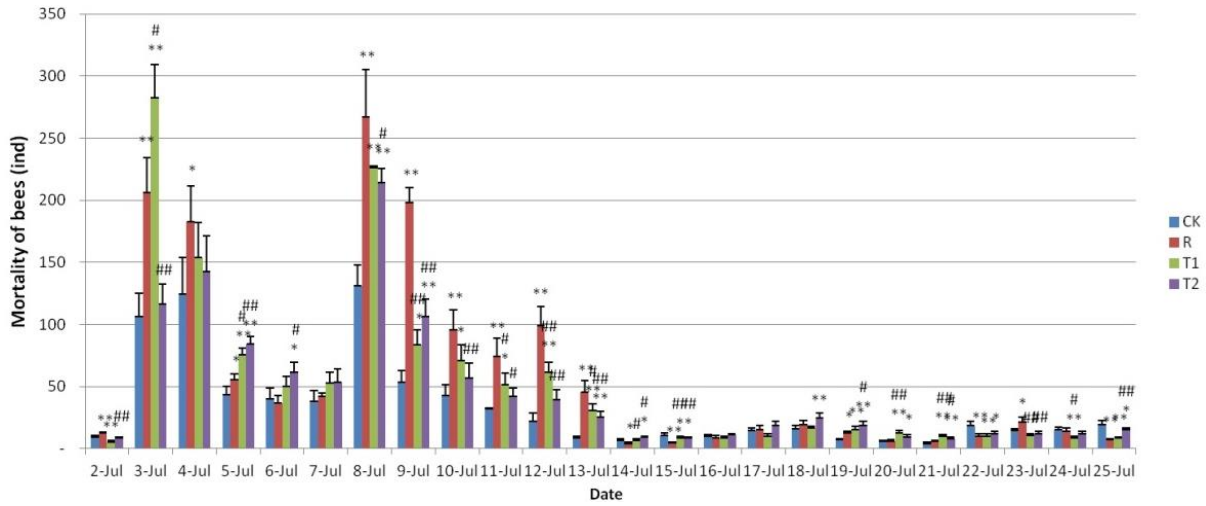


**Figure 1.** Change of individual (ind) bee mortality in each group with time from the beginning of exposure to the end of the test. CK: water control; R: reference substance treatment, dimethoate 600 g a.i./ha applied on July 8; T1: sulfoxaflor treatment, 75 g a.i./ha applied on July 1 and July 8;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