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Effect of fenpropathrin on the viability and homing ability of worker bees *Apis mellifera*

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ABSTRACT

To explore the effect of fenpropathrin on survival and homing ability of honeybees *Apis mellifera* L., the newly emerged honeybee workers (< 12 h old) were randomly divided into 4 groups with 3 replicates in each group. Fenpropathrin (1/2 LD_{50}, 1/4 LD_{50}, 1/8 LD_{50} and 0% LD_{50}) was added on the thorax of the bees. The viability of worker bees and their homing rate at 1 km distance away from colonies were analyzed, and the expression levels of two memory related genes (*GluRA* and *Nmdar 1*) in 20-day–old worker bees were also quantified. Overall, the lifespan and homing rates were significantly decreased with the increase of fenpropathrin dose ($P < 0.05$), but there was no significant difference between the least group (1/8 LD_{50}) and the control group (0% LD_{50}) ($P > 0.05$). The relative expression of *Nmdar1* was decreased significantly with the increasing doses of fenpropathrin and the lower expression level of *Nmdar* 1 was found in the fenpropathrin-treaded groups. The expression level of *GluRA* of workers in 1/8 LD_{50} group and the control group were significantly higher than that in 1/2 LD_{50} group and 1/4 LD_{50} group ($P < 0.05$), whereas the expression level of *GluRA* of workers in 1/4 LD_{50} group was significantly higher than that in 1/2 LD_{50} group ($P < 0.05$), and there is no significant difference between 1/8 LD_{50} group and the control group ($P > 0.05$). In conclusion, the use of fenpropathrin for agricultural crops may show negative influence on the viability and homing ability of worker bees *Apis mellifera* L.

Introduction

Honeybee is a eusocial insect species. They are one of the most important pollinator for plants. However, their foraging activities can be negatively influenced by many kinds of factors including pesticides and parasites. Pesticides are important agricultural tools to control insect pests in the field, however, extensive usage of pesticides are threatening the pollinator populations (Sally and Geraldine, 2013). Researches have showed that the pesticides are one of main factors dramatically affecting honeybee foraging behavior and potentially contributing to honeybee colony losses (Underwood and Vanengelsdorp, 2007; Ei-Hassani et al., 2008; Allouane et al., 2009; Cresswell and Thompson, 2012; Rundlöf et al., 2015; Tan et al., 2013, 2014, 2015). Pesticide-polluted plants offer harmful nectar and pollen to foragers and affect the honeybee colony (Girolami et al., 2009; Pohorecka et al., 2012). A previous study showed that honeybees prefer to collect the food containing neonicotinoid pesticides (Kessler et al., 2015) and the neonicotinoid and pyrethrins pesticides impair the survival, individual health, colony growth, queen reproductively, foraging activity and olfactory learning of honeybees (Whitehorn et al., 2012; Sally and Geraldine, 2013; Tan et al., 2013, 2014, 2015; Raine and Gill, 2015; Rundlöf et al., 2015; Stanley et al., 2011).

The fenpropathrin is one of synthetic pyrethroid pesticides that are widely used as broad spectrum insecticides in cotton, cereals, vegetable and other crops (Zuo, 2008). Many researchers have reported that insecticides, such as neurotoxic pyrethroids, elicit various adverse effects in insects when used in agricultural production (Yousef, 2010; Zhang et al., 2010; Joshi et al., 2011). Honeybee foraging and homing activities could be dramatically influenced while exposing in neurotoxic pyrethroids (Dame et al., 1995; Decourtye et al., 2004), reflecting that these pesticides can enormously harm honeybee physiological health, especially its cognitive physiology. As a high neurotoxic and efficient insecticide, the fenpropathrin has been widely used in killing mites and pests for crops (Tan et al., 2001). Honeybees, therefore, are acutely exposed in this pesticide while foraging contaminated nectar and pollen from crops. A previous investigation revealed the fenpropathrin had much higher toxicity on honeybees compared to fungicides and bacteria (Rasuli et al., 2015), and other scientists appealed to stop using this pesticide when plants are blossoming (Riedl et al., 2006). However, it is still unclear whether this pesticide could affect the viability and homing

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ability of honeybees. Homing ability is associated with learning and memory, related genes expression of *N-methyl-D-aspartic acid receptor 1* (*Nmdar 1*) and *Glutamate receptor A* (*GluRA*) (Kucharski et al., 2007; Zachepilo et al., 2008).This study aims to determine the effects of three low doses of fenpropathrin on the viability, homing ability and the relative gene expression levels of *GluRA* and *Nmdar1* of worker bees in western honeybee *Apis mellifera* L.

Methods

Experiment design

Three *Apis mellifera* L. colonies used in this study were kept in the apiary of Jiangxi Agricultural University under normal living conditions. The queen of each colony was restricted to laying eggs in one comb, and was removed to another comb after 24 h. 19 days later, the brood frames with capped cells were transferred to an incubator (34.5 ± 0.5 °C at 70% humidity) to get emerging worker bees. Newly emerged worker bees (< 12 h old) from each colony were randomly divided into 4 treatment-groups, each of the four treatment groups had three replicate cages with 300 bees per cage.

Fenpropathrin was provided by Zhejiang Weierda Chemical Co., Ltd. and was mixed thoroughly into acetone to obtain the desired concentrations. To identify whether the dose of fenpropathrin influence the survival and homing ability of *Apis mellifera* L., the bees of the four treatments were dripped 1.5 μL fenpropathrin solution (Contain 1/2 LD_{50}, 1/4 LD_{50}, 1/8 LD_{50} and 0% LD_{50} fenpropathrin) on the thorax by contact method, respectively (Ei-Hassani et al., 2008; Zhou et al.,2014). To do so, each honeybee of the 4 treatments was caught in the cage and maintained with a forceps while 1.5 μL of the solution was applied to the thorax using a micropipette with a tip. After the drop disappeared, the honeybee was released into a new cage where the treated bees with same solution were gradually collected.

The survival experiments

The treated bees in each replicate per group were assigned to wooden cages with mesh on one side, respectively, which then were transferred to an incubator (34.5 ± 0.5 °C at 70% humidity), with 50% sucrose solution. The number of dead bees was counted and cleared away every day until all the bees sacrificed.

The homing ability experiments

Newly emerged worker bees were treated with 1.5 μL of fenpropathrin under four different concentrations as above and marked with different colors of paint (San Ling Co., Ltd., Japan). Each treatment had three biological replicates and each replicate contained approximately 300 bees. Afterwards, they were returned into their natal colonies. 20 days later, > 30 marked bees with each color were captured from each colony and immobilized on ice, so that a RFID tag with known id number could be glued to each bee's thorax. Twenty tagged bees of each replicate in each treatment were kept in cages with *ad libitum* access to 50% sucrose solution. Cages were transported to the respective release site in dark styrofoam containers 1 km away from the hive and were opened at one side. Upon return to the hive, the bees' identity and homing time were recorded by the RFID receivers at the hive entrance (Pahl et al., 2011; He et al., 2013).

The relative expression level of genes related to learning and memory

To evaluate the effect of fenpropathrin on the relative expression level of genes (*GluRA* and *Nmdar 1*) related to honeybee learning and memory processes, newly emerged worker bees were treated and marked with different colors as above. After returning into their natal colonies for 20 days, heads of 3 bees were taken as a sample and 3 samples were got in each replicates of the four groups. The relative expression level of *GluRA* and *Nmdar 1* in heads of 20-day worker bees was quantified. The RT-qPCR was performed using CFX96 with the primers listed in Table 1 and the *GAPDH* gene was used as an internal control. All samples were analyzed in triplicate. The relative gene expression levels of these genes were calculated using the $2^{-\Delta\Delta Ct}$ comparative CT method (Schmittgen and Livak, 2008).

Table 1
Gene-specific primers used in real time quantitative PCR.

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
GluRA	ACTCTGTTCGTCTGTGGGGTG	TTCGTTAGAAGGGCAGCGTA
Nmdar1	GTATTTCCGTCGCCAAGTC	TGTAAACCAATCCCATAGCCA
GAPDH	GCTGGTTTCATCGATGGTTT	ACGATTTCGACCACCGTAAC

Statistical analyses

SPSS 17.0 software was used to analyze the survival of the experimental data by Kaplan-Meier method, and the ANOVA test followed by fisher's LSD test was used for statistical analysis of lifespan and homing rates. The analysis of genes expression was performed with Duncan's multiple range tests using SPSS 17.0. Data are means ± *SE*.

Results

Effect of fenpropathrin on the lifespan of worker bees Apis mellifera L.

The survival curve of worker bees showed that the survival time of 1/2 LD_{50} group was significantly lower than that of other groups. Most of the workers in 1/2 LD_{50} group died nearly at the age of 15 days, while most of the workers in other groups died nearly at the age of 20 days (Fig. 1). The average lifespan of worker bees decreased significantly with the increase of fenpropathrin doses ($P < 0.05$). The average lifespan of worker bees in 1/8 LD_{50} group and the control group were significantly longer than that in 1/2 LD_{50} group and 1/4 LD_{50} group ($P < 0.05$). The lifespan of worker bees in 1/4 LD_{50} group were significantly longer than those of 1/2 LD_{50} group ($P < 0.05$). There was no significant difference between 1/8 LD_{50} group and the control group ($P > 0.05$) (Fig. 2).

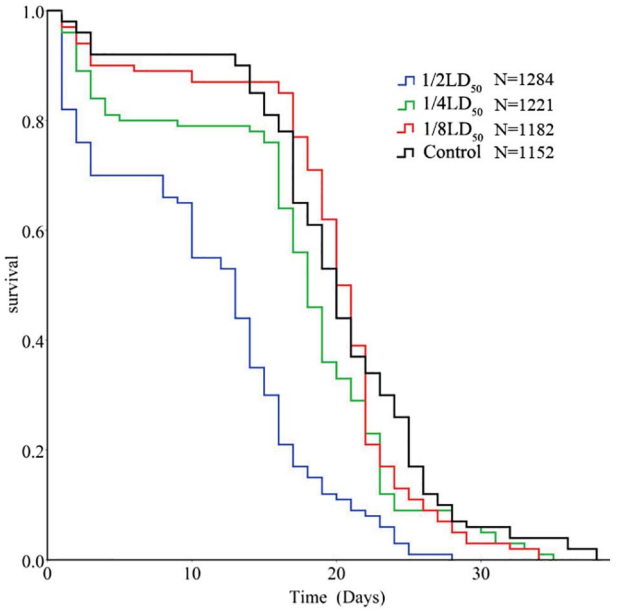

Fig. 1. The survival curve of workers bees *Apis mellifera* L. treated with different doses of fenpropathrin.

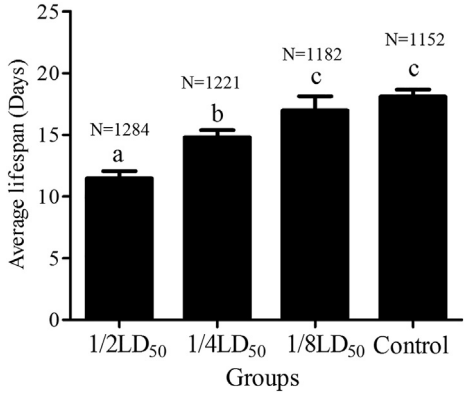

Fig. 2. The effect of fenpropathrin on the lifespan of worker bees *Apis mellifera* L. Different letters on top of bars indicate significant difference ($P < 0.05$) between groups. The same notation is used in Figs. 3 and 4.

Effect of fenpropathrin on the homing rates of worker bees Apis mellifera L.

The effect of fenpropathrin on the homing rates showed a similar trend as lifespan (Fig. 3). The homing rates of worker bees in 1/8 LD_{50} group and the control group were significantly higher than that in 1/2 LD_{50} group and 1/4 LD_{50} group ($P < 0.05$), and that in 1/4 LD_{50} group was significantly higher than that in 1/2 LD_{50} group ($P < 0.05$), but there was no significant difference between 1/8 LD_{50} group and the control group ($P > 0.05$).

The effect of fenpropathrin on the expression of genes related to learning and memory of worker bees Apis mellifera L.

The expression level of *Nmdar* 1 significantly decreased with the increase of the fenpropathrin doses, and it was significantly higher in the control group compared to that in the fenpropathrin-treated groups ($P < 0.05$) (1/2 LD_{50}, 1/4 LD_{50} and 1/8 LD_{50} group). The expression level of *GluRA* in workers of 1/8 LD_{50} group and the control group were significantly higher than those in 1/2 LD_{50} group and 1/4 LD_{50} group ($P < 0.05$), while it was significantly higher in 1/4 LD_{50} group compared to that in 1/2 LD_{50} group ($P < 0.05$). There was no significant difference between 1/8 LD_{50} group and the control group ($P > 0.05$) (Fig. 4).

Discussion

Neurotoxic pesticides are threatening the global honeybee species, and are considered as one of major factors inducing the high ratio of honeybee death (Oldroyd, 2007; Frazier et al., 2008). Honeybees are exposed to these pesticides when forage on polluted crops (Tan et al., 2001). They also touch these insecticides in bee hives when some of these pesticides are used for killing *varroa* mites (Oldroyd, 2007). For example, pyrethroids such as fluvalinate not only kill the *Varroa* mites, but also affects honeybees within the colonies (Haarmann et al., 2002; Decourtye et al., 2005; Frost et al., 2013). The evaporation of fluvalinate causes bees to contact the pesticides and pollutes the bee's food. Another pyrethroid, deltamethrin not only decreases honeybee learning and memory capabilities, but also induces motor incoordination or muscular troubles (Dame et al., 1995). Further, fluvalinate delays honeybee larval development as well as adult longevity (Wu et al., 2011). Fenpropathrin, one of the neurotoxic pesticides, is widely used in crops, but few studies have examined its effect on survival and homing ability of worker bees. Our results showed that low dose fenpropathrin (1/4 LD_{50} to 1/2 LD_{50}) significantly reduced the survival ratio of workers *Apis mellifera* L., which indicated that this pesticide offers severe adverse effects on honeybee health. Furthermore, the results of homing experiment showed that lower concentration of fenpropathrin could considerably affect the homing ability of honeybee. Previous studies have reported that pesticides such as fipronil, thiamethoxam and acetamiprid significantly reduced honeybee longevity, foraging activities and homing rate (Ei-Hassani et al., 2005, 2008). This study showed that a lower dose (1/4 LD_{50}) of fenpropathrin could offered a dramatic adverse effect on honeybee survival and homing ability. This suggests that adverse effects induce by lower doses of pesticide (lower than median lethal dose) on honeybee should not be overlooked in agricultural application.

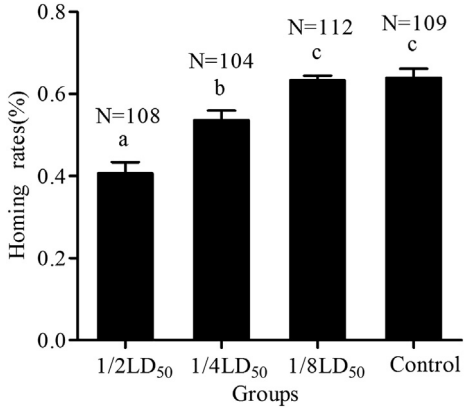

Fig. 3. The effect of fenpropathrin on homing rates of worker bees released at 1000 m away from the hive.

Previous studies revealed that the voltage-gated sodium channel of insect is the primary target of insecticides including pyrethroids which can interact with a few target molecules such as neurotransmitter receptors, ion channels and membrane transport processes to damage the physiological process and cognitive function (Sattelle and Yamamoto, 1988; Grünewald et al., 2004). In this study, we supposed that the insecticide fenpropathrin may reduce the flying ability of forager bees by targeting the voltage-gated sodium channel in flying muscles. Moreover, the homing ability also depends on the spatial memory and navigation capabilities. The *GluRA* is a metabotropic glutamate receptor present in the central nervous system of honeybees (Kucharski et al., 2007), which can directly affects the learning and memorizing capability of honeybees. Our results showed that the expression level of memory related genes significantly decreased with the increase of fenpropathrin dose. This suggests that fenpropathrin can affect the nervous system of honeybees and impact physiological processes of related to memory and learning. Whereas, another receptor protein, *Nmda1* related to learning and memory of honeybees is found in the brain Kane mushroom body cells honeybee (Zachepilo et al., 2008). When the bees touch a certain amount of fenpropathrin, the expression level of the gene *Nmda1* gets affected and the learning- memory ability declines. The genes *GluRA* and *Nmda1* were down-regulated in 1/2 LD_{50} group and 1/4 LD_{50} group compared to 1/8 LD_{50} and control group, indicating that 1/4 LD_{50} dose of fenpropathrin would impair the nervous system and brain Kane mushroom body cells of honeybees. Learning impairment may decrease the homing ability and the lifespan of worker bees *Apis mellifera* which will reduce colony fitness and health. It is still unclear whether the muscular troubles or troubles in information storage and retrieval induced by the fenpropathrin resulted in low level of homing rate, we speculate that the fenpropathrin could trigger muscular and neural troubles similar as the deltamethrin in a previous study (Dame et al., 1995). However, it still needs a further investigation.

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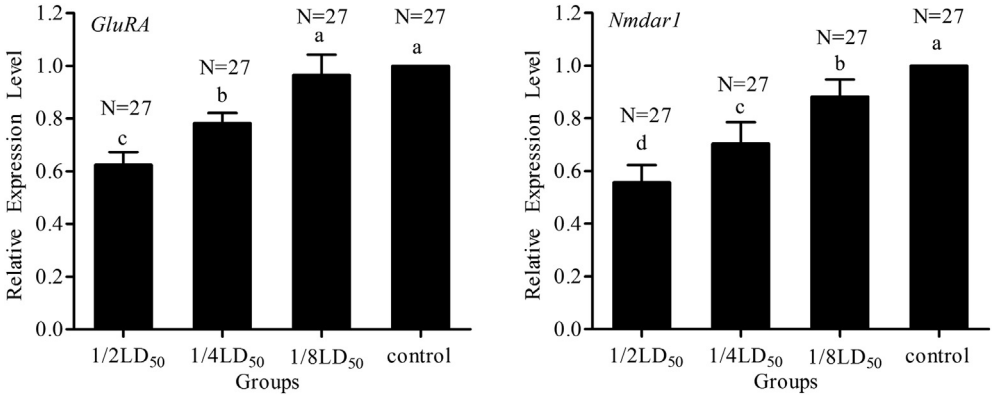

Fig. 4. The effect of fenpropathrin on the expression of genes related to learning and memory of worker bees *Apis mellifera* L.

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Conflict of interest

No conflict of interest exits in the submission of this manuscript.

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