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Sublethal acetamiprid doses negatively affect the lifespans and foraging behaviors of honey bee (*Apis mellifera* L.) workers

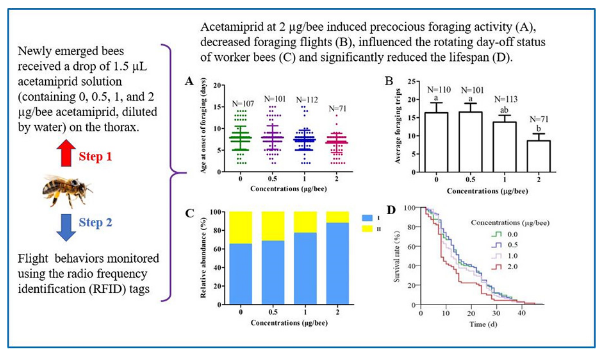
Jingliang Shi [a,b], Heyan Yang [a], Longtao Yu [a], Chunhua Liao [c], Yao Liu [a], Mengjie Jin [a], Weiyu Yan [a], Xiao Bo Wu [a,*]

[a] *Honeybee Research Institute, Jiangxi Agricultural University, Nanchang 330045, PR China*
[b] *College of Environmental Science and Engineering, Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, Nankai University, Tianjin 300350, PR China*
[c] *Guangyuan City Animal Husbandry and Seed Management Station, Guangyuan, 628017, Sichuan, PR China*

HIGHLIGHTS

- Worker bees exposed to 2 μg/bee acetamiprid induced precocious foraging activity and shortened the lifespan.
- Worker bees exposed to 2 μg/bee acetamiprid heavily decreased the workload throughout their lifetime.
- Excessive day-off rotation of worker bees exposed to 2 μg/bee acetamiprid is firstly reported.

GRAPHICAL ABSTRACT



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ABSTRACT

The neonicotinoid insecticide acetamiprid is applied widely for pest control in agriculture production. However, little is known about the effects of acetamiprid on the foraging behavior of nontarget pollinators. This study aims to investigate effects of sublethal acetamiprid doses on lifespans and foraging behaviors of honey bees (*Apis mellifera* L.) under natural swarm conditions. Newly emerged worker bees of each treatment received a drop of 1.5 μL acetamiprid solution (containing 0, 0.5, 1, and 2 μg/bee acetamiprid, diluted by water) on the thorax respectively. Bees from 2-day-old to deadline were monitored on foraging behaviors involving the age of bee for first foraging flights, rotating day-off status and the number of foraging flights using the radio frequency identification (RFID) system. We found that acetamiprid at 2 μg/bee significantly reduced the lifespan, induced precocious foraging activity, influenced the rotating day-off status and decreased foraging flights of worker bees. The abnormal behaviors of worker bees may be associated with a decline in lifespan. This work may provide a new perspective into the neonicotinoids that accelerate the colony failure.

1. Introduction

Honey bee (*Apis mellifera* L.) as an important pollinator not only provides nutritious bee products for its efficient foraging capability, but also plays a vital role in agricultural production and in maintaining ecological balance (Klein et al., 2006). However, a large number of scientific investigations have reported the heavy losses of bee colonies across the world (Potts et al., 2010; Santos et al., 2014; Antúnez et al., 2016). A sharp decline of honey bees seriously restricts the development of bee industry and may cause plant pollination crisis (Clermont et al., 2015). In recent years, a variety of factors linked to declined bees, such as parasitic mites, pathogens, pesticides, habitat loss, poor nutrition and magnetic field (Naug, 2009; Ferrari, 2014; Goulson et al.,

* Corresponding author.
E-mail address: wuxiaobo21@163.com (X.B. Wu).

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2015; Sánchez-Bayo et al., 2016; Abbo et al., 2016; Steinhauer et al., 2018), have been studied to determine causes of colony losses. Moreover, interactions between various factors may generate synergies on honey bee populations (Goulson et al., 2015; Chambers et al., 2019).

To date, although exact causes of bee declines is complex and incomprehensible, the widespread use of pesticides is widely considered to be a major factor (Sánchez-Bayo et al., 2016). Pesticides have been applied broadly in agriculture for pest control, and bees are susceptible to be poisoned (Thompson et al., 2010). Previous studies found that bees treated with insecticides can impair the lifespan, colony growth, and foraging behavior of honeybees (Thompson et al., 2005; Yang et al., 2008; Tan et al., 2014; Shi et al., 2019a). Remarkably, the acute toxicity of insecticides to honey bees is well identified usually through determining the LD_{50} or LC_{50} values under laboratory conditions (Iwasa et al., 2004; Laurino et al., 2011; Jacob et al., 2019a). Nevertheless, bees in many cases are exposed to sublethal doses of insecticides. Numerous studies have reported the potential threats of sublethal insecticides doses to honey bees, such as affecting the development of bee larvae and queen bees (Wang et al., 2017; Wu-Smart and Spivak, 2016), causing metabolic disorders and abnormal foraging behaviors of honey bees (Schneider et al., 2011; Cook, 2019; Stanley et al., 2016). Therefore, it is of greater practical significance to assess the effect of sublethal doses of insecticides on honey bees.

Among the various insecticides, including organophosphates, neonicotinoids, pyrethroids, and carbamates, the neonicotinoids are the most widely used insecticides around the world (Lin et al., 2014). Because compared with other insecticides, neonicotinoids have higher selectivity for insects versus mammals (Tomizawa and Casida, 2004). Neonicotinoids can act on insect nicotinic acetylcholine receptors (nAChRs) and interfere with the conduction of its nervous system, then cause the insect to die for paralysis (Bicker, 1999). Unfortunately, pollinators as the nontarget insects like targets have been chronically suffered from the deleterious effects of neonicotinoids, especially for bee species. Furthermore, bee genomes code significantly deficient detoxifying enzymes compared with other insect genomes (Claudianos et al., 2006; Johnson et al., 2010). Therefore, neonicotinoids at sublethal doses can seriously affect the survival, foraging activity and olfactory memory (Hassani et al., 2008; Schneider et al., 2011; Abdel–Kader et al., 2017; Shi et al., 2019a).

Recently, three highly toxic neonicotinoids were banned since 2013 (Godfray et al., 2014), while acetamiprid is currently the mainly one available neonicotinoid in EU for its low acute toxicity on bees (Iwasa et al., 2004; Yang et al., 2019). However, the chronic sublethal effects of acetamiprid on honey bees should not be ignored since high acetamiprid residue levels in pollen and beeswax have been frequently detected (Mullin et al., 2010; Jabot et al., 2015). Acetamiprid is a primary member of neonicotinoid insecticides with broad-spectrum characteristic and widely used in the tea, vegetables, fruit trees, flowers and other plants for pest control (Zhou et al., 2006). Meanwhile, bees are frequently exposed to pesticide residues by collecting nectar and pollen from flowering plants which are treated with pesticides. Most strikingly, beehives in many cases are placed near blooming crops for improving the collection efficiency and subsequently much honey profit. Therefore, sprayed pesticides will easily enter the beehive with the form of fog. All individuals especially for younger bees in the colony are inevitably contact the pesticides. Sublethal doses of acetamiprid negatively influence the cognition and long-term memory of honey bees (Hassani et al., 2008; Aliouane et al., 2010). Moreover, when acetamiprid residue in queen cells is over 100 μg/kg, it will generate negative effects on queen rearing (Shi et al., 2019b). A recent research found nearly half pesticides in combination with acetamiprid acted synergistic effects on honey bees (Wang et al., 2020). However, there is still data gap for the risk assessment of chronic toxicity about acetamiprid to honey bees (EFSA, 2016), especially for effects of acetamiprid on foraging behavior of honey bees have rarely been previously investigated, though adverse effects of imidacloprid or other neonicotinoid compounds on the foraging behavior of worker honey bees were frequently reported (Yang et al., 2008; Schneider et al., 2011; Tan et al., 2014). For this reason, the present study in detail assessed how sublethal doses of acetamiprid affect the foraging performance of *A. mellifera* workers, which containing individual bees performed their first foraging flights, rotating day-off status and the number of foraging flights. Furthermore, the lifespan of worker bees exposed to acetamiprid was also examined. The above indicators were achieved with the help of radio frequency identification (RFID) system. RFID system can be used to systematically study the biological characteristics of social bees and dynamically monitor the target bees at the entrance of the hive as bees come and go (He et al., 2013; Henry et al., 2015).

Former studies proposed and used the newly emerged bees for comparative physiological, developmental, and host-pathogen studies (Erban et al., 2016, 2019a). In this study, the lifespan and foraging behavior of newly emerged worker bees from emergence to death were documented with acetamiprid exposure in natural swarm conditions using RFID system. In addition, this is the first study to investigate whether exposed to acetamiprid of worker bees can change the rotating day-off status in the foragers. Honey bees from the same colony exists a rotation day-off system which is similar to humans (Moore et al., 1989; Klein and Seeley, 2011), proper day-off rotation is helpful to improve work efficiency, but excessive day-off rotation is a kind of go-slow phenomenon. Here, we speculate that the foraging behaviors involving first foraging flights, rotating day-off status and the number of foraging flights would be affected by exposure to acetamiprid.

2. Materials and methods

2.1. Insects

Experimental honey bee (*Apis mellifera* L.) colonies were reared at the Honeybee Research Institute, Jiangxi Agricultural University, Nanchang, China (28.46° N, 115.49° E). Selected bee colonies were strong and healthy colonies that were not threatened by parasitic mites or other parasites and had no prior exposure to pesticides. All experimental work was conducted from May to July of 2019.

2.2. Radio frequency identification setup

Two empty hives (standard Langstroth hive) were selected to setup the radio frequency identification (RFID). A one-way tunnel for bees (one in and one out) at the entrance of the hive was artificially designed. Two tunnels leading in and out of the hive, respectively, were equipped with four antennae connected to an RFID reader (Invengo XC-RF807) so that bees come and go could be sensed by antennae, and documented by the computer through a network cable (He et al., 2013; Colin et al., 2019). Then, we set the network ID by computer and named the electronic tags. The next step was to open the reader after the device was connected. Furthermore, tags glued to a small stick were used to test the induction performance in advance. Further experiments were started with good induction performance. 3-frame with bees containing a queen were put into the RFID device ahead a week in order to adapt bees to flight.

2.3. Contact exposure to sublethal doses of acetamiprid

When bees are about to emerge, frames with capped brood from two different colonies were transferred into an incubator waiting for emergence. Meanwhile, we set three concentrations (333, 667, and 1333 mg/L) of acetamiprid for bees' treatment according to the field-realistic concentrations of acetamiprid provided by the manufacturer (50–500 mg/L) (Shi et al., 2019a). Distilled water was used to dissolve acetamiprid and obtain desired concentrations, and distilled water was used as a control. The acetamiprid as a commercial product was bought from Jiangxi Heyi Chemical Co., Ltd., which contains 70%

acetamiprid, bentonite (packing materials, 12%), ammonium chloride (disintegrating agent, 10%), ZX-D9 (dispersing agents, 4%), naphthalene-sulfonic acid formaldehyde condensate (dispersing agents, 2%), and M (wetting agent, 2%) (Zhang, 2008). The prepared acetamiprid solutions were stored in 4 °C refrigerator for contact exposure of bees. We collected approximately 80–100 newly emerged worker bees in each treatment group in a single colony within 12 h, bees of each treatment received a drop of 1.5 μL acetamiprid solution (containing 0, 0.5, 1, and 2 μg/bee) on the thorax respectively, and the sublethal doses were set depending on previous reported LD_{50} of acetamiprid on honey bees (7.1 μg/bee) in contact exposure (Iwasa et al., 2004). After treatment with acetamiprid solution, all bees were put into an incubator for restoration (34 °C at 70 ± 5% humidity) and fed 50% sucrose water and pollen. In the second day, bees (2-day-old worker bees) in dead or poor state (motor disorder) were eliminated, and only the remaining healthy bees would be monitored. A study by Shi et al. (2019a) reported that dose of acetamiprid over 1 μg/bee significantly reduced the lifespan of bees, especially the death caused by acute toxicity on the first day.

2.4. Bee tagging and dynamic monitoring

2-day-old worker bees from each treatment group in good condition were captured into 10 mL centrifuge tubes and immobilized on ice to chill 3–5 min, because research has found that ice-chilling did not affect the memory of honey bees compared to other cold immobilization methods (Frost et al., 2011). Then a numbered electronic tag was quickly glued to the thorax of the bee until all tags were glued to the corresponding bees. Bees were put back into the incubator for 2 h to recover, and all bees were introduced into the hive for RFID monitoring (He et al., 2013).

2.5. Statistical analysis

GraphPad Prism 5 software was used to draw all data figures, and chi-square test was performed on the percentage of rotating day-off bees among groups using GraphPad Prism 5 software. The SPSS17.0 software was used to analyze the survival between different groups by Kaplan-Meier method. The boxplot method in descriptive statistics of SPSS17.0 was used to remove the abnormal values (values over mean ± 3 times standard deviation were abnormal) to meet normality before we performed the variance (ANOVA) to analyze the age at onset of foraging (bees leaved the hive for >5 min were regarded as a foraging activity (Tian et al., 2014)), and the average foraging trips; meanwhile, data from four groups was consistent with homogeneity of variances, when $P < 0.05$, we used the ANOVA test followed with Fisher's LSD test to determine whether there were any differences among different groups.

$$\text{Percentage of rotating day-off (\%)} = \frac{\text{Rotating day-off bees}}{\text{Total monitoring bees}} \times 100\%$$

(Tian et al., 2014)

$$\text{Average foraging trips} = \frac{\text{Lifetime number of trips}}{\text{Total monitoring bees}}$$ (Colin et al., 2019)

3. Results

3.1. Precocious foraging activity of worker bees induced by acetamiprid

The age at onset of foraging in the 2 μg/bee group was significantly lower than those in the 0.5 μg/bee group and 0 μg/bee group ($P_{(0,2)} = 0.002$); $P_{(0.5, 2)} = 0.001$), respectively, while there was no significant difference between 1 μg/bee group and 2 μg/bee group ($P > 0.05$) (Fig. 1, Table 1). Remarkably, the age at onset of foraging showed no significant difference among 0 μg/bee group, 0.5 μg/bee group and 1 μg/bee group ($F_{2,317} = 1.341$, df = 2, $P > 0.05$). Honey bee workers exposed to

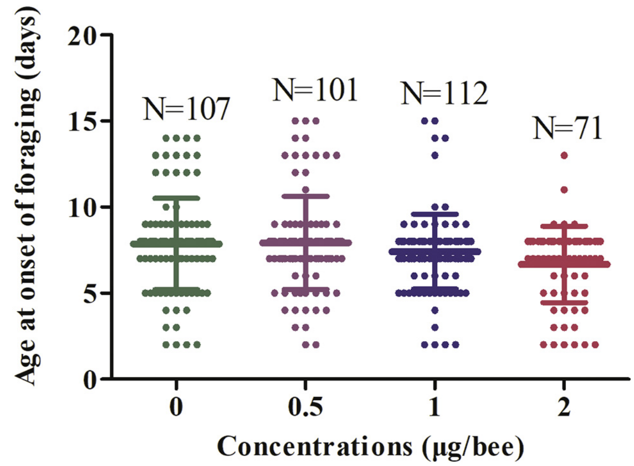

Fig. 1. Acetamiprid induced precocious foraging activity of worker bees *Apis mellifera*. Age at onset of foraging (days) of worker bees. The X-axis represents treated acetamiprid concentrations of each group. *N* values denote number of worker bees.

2 μg/bee acetamiprid solution were 1.18 days on average earlier than 0 μg/bee group on the age at onset of foraging, and bees in 2 μg/bee group began to forage on 6.68 days ($F_{3,387} = 4.436$, df = 3, $P = 0.004$; Fig. 1). The results indicated that worker bees exposed to over 2 μg/bee acetamiprid will induce precocious foraging activity.

3.2. Excessive day-off rotation of worker bees by acetamiprid

Honey bee workers emerged day-off behavior were defined as rotating day-off bees, while bees on continuously working days throughout their life were defined as continuously working bees, and we counted bees that left the hive for more than 5 min as a foraging flight (Calderone and Page, 1988). With the increasing concentration of acetamiprid, the proportion of rotating day-off bees was gradually increased (Fig. 2, Table 1). Unbelievably, the rotating day-off proportion in 2 μg/bee group was up to 87.50%, significantly higher than that in the other three groups ($\chi 2 = 13.66$, df = 3, $P = .003$), and increased 34.62% compared with the 0 μg/bee group. However, there were no significant differences among 1 μg/bee, 0.5 μg/bee and 0 μg/bee groups ($\chi 2 = 4.20$, df = 2, $P = .123$). As mentioned above, worker bees treated with sublethal acetamiprid doses exhibited higher proportion of day-off rotation.

3.3. Worker bees exposed to acetamiprid decreased the workload throughout their lifetime

The frequency of bees foraging activity is an important parameter to characterize the workload of bees. The number of foraging trips of each bee was counted and analyzed (Fig. 3, Table 1). Bees in the 2 μg/bee group performed, on average, 7.72 and 7.91 less foraging trips than those in the 0 μg/bee group and 0.5 μg/bee group, respectively ($P_{(0,2)} = 0.030$; $P_{(0.5, 2)} = 0.029$). There was no significant difference between 2 μg/bee group and 1 μg/bee group ($P > 0.05$), similar results between 0 μg/bee, 0.5 μg/bee and 1 μg/bee groups. Therefore, sublethal dose of acetamiprid decreased the workload of worker bees.

3.4. The lifespan of worker bees shortened by acetamiprid

Marked worker bees of each group were monitored from 2-day-old to the last day (missing records from that day) in natural swarm conditions, and dates of the last record were regarded as the lifespan of bees. Results showed that the average lifespan of 2 μg/bee group was significantly lower than those in 0 μg/bee group and 0.5 μg/bee group ($\chi 2 = 8.491$, df = 2, $P = .014$), while there was no statistical difference

Table 1
Effects of different acetamiprid doses on the age at onset of foraging, average foraging trips and rotating day-off proportion in *Apis mellifera*.

Groups (μg/bee)	Age at onset of foraging (days)	Average foraging trips (trips)	proportion of rotating day-off bees (%)
0.0	7.860 ± 2.647[a]	16.346 ± 2.763[a]	65.00
0.5	7.921 ± 2.697[a]	16.545 ± 2.377[a]	68.32
1.0	7.411 ± 2.175[ab]	13.779 ± 1.868[ab]	76.99
2.0	6.676 ± 2.215[b]	8.636 ± 1.954[b]	87.50

Values (mean ± *SD*) of the age at onset of foraging and the average foraging trips in the same column with different letters are significantly different (LSD test, $P < 0.05$), the proportion of rotating day-off bees among four groups were analyzed using chi-square test.

between 1 μg/bee group and 2 μg/bee group ($\chi2 = 2.974$, df = 1, $P = .085$), and among 0 μg/bee, 0.5 μg/bee and 1 μg/bee groups ($\chi2 = 1.092$, df = 2, $P = .579$) (Table 2). Over half of worker bees in the 2 μg/bee group died approximately at the age of 8 days, 7 days earlier than that of 0 μg/bee group and 0.5 μg/bee group (Table 2, Fig. 4). In all, over 2 μg/bee acetamiprid can seriously damage the survival ability of honey bees.

4. Discussion

All experimental data were well documented in swarm conditions using RFID technology, the survival and foraging behavior of honey bee individuals exposed to sublethal acetamiprid doses were monitored throughout their life. As much research verified the high toxicity of the nitro-substituted compounds mainly including imidacloprid, thiamethoxam and clothianidin on honey bees or other bee species, while lower acute toxicity of cyano-substituted neonicotinoids such as acetamiprid (Tome´ et al., 2012; Tavares et al., 2019; Tadei et al., 2019; Jacob et al., 2019b). We hypothesize that exposure to sublethal doses of acetamiprid may cause behavior disorder of honey bees. Furthermore, previous studies have not yet provided the information about the effect of acetamiprid on worker bee foraging behavior. In this study, our results showed negatively adverse effects of sublethal acetamiprid doses on first foraging flights, rotating day-off status, average foraging flights and lifespans of *A. mellifera* worker bees. Results of the survival showed that the average lifespan of worker bees in 2 μg/bee group was significantly lower than those in 0.5 μg/bee and 0 μg/bee groups, while there were no statistical differences between 1 μg/bee group and any other groups (Fig. 4, Table 2). However, a recent study under the laboratory condition showed that the average lifespan of worker bees in 1 μg/bee group was significantly higher than that in 2 μg/bee group, but significantly lower than that in 0 μg/bee group and 0.5 μg/bee group (Shi et al., 2019a). The main reason is that there are certain differences in the experimental conditions, one was conducted in natural colonies, and the other was in laboratory. In other words, there are certain differences between the environment and food. Similar situations were frequently found in other studies mainly due to varied methodology of investigations (Cresswell, 2011). Another reason is that bees in dead or poor state were eliminated before the monitoring, and only the remaining healthy bees were monitored, given hardly acquiring the foraging flights of poor state bees. Overall, bees exposed to acetamiprid at a dose >2 μg/bee would negatively affect the lifespan of worker bees.

Results on the first foraging flights of worker bees showed that the age at onset of foraging in 2 μg/bee group was about 15% earlier than that in 0 μg/bee group and 0.5 μg/bee group (Fig. 1, Table 1), indicating that acetamiprid could promote the early development of worker bees into foragers. This result was similar to the finding by Colin et al. (2019) that imidacloprid could induce precocious foraging behavior of honey bees. We compared this result to the lifespan, one possible explanation is that exposure to acetamiprid can cause the younger bees to start foraging earlier in their adult life, thus shortening their lifespans. Furthermore, the study of Tian et al. (2014) showed that the precocious foraging of young bees would significantly reduce their lifespans.

The proportion of rotating day-off bees in the normal healthy colony was approximately 64%, and the lifespan of rotating day-off bees was significantly higher than that of continuously working bees (Tian et al., 2014). In the present study, the proportion of rotating day-off bees in the 0 μg/bee group was 65%, but the rotating day-off ratio of worker bees also showed an increasing trend with the increasing used doses of acetamiprid, and the proportion of rotating day-off bees in the 2 μg/bee group reached to 87.50% (Fig. 2, Table 1). The reason may be that acetamiprid poses adverse impact on honey bee physical

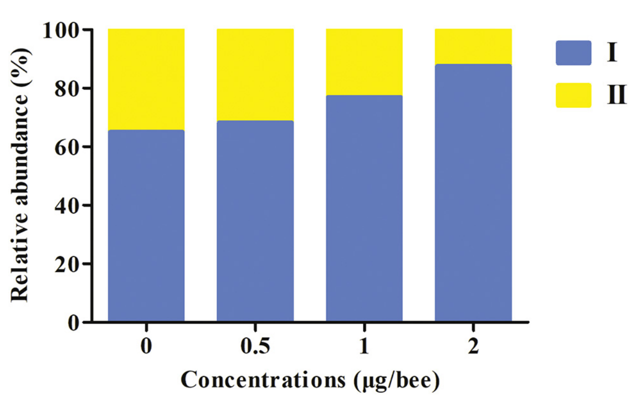

Fig. 2. Acetamiprid changed the proportion of rotating day-off bees and continuously working bees. I: Rotating day-off bees; II: Continuously working bees.

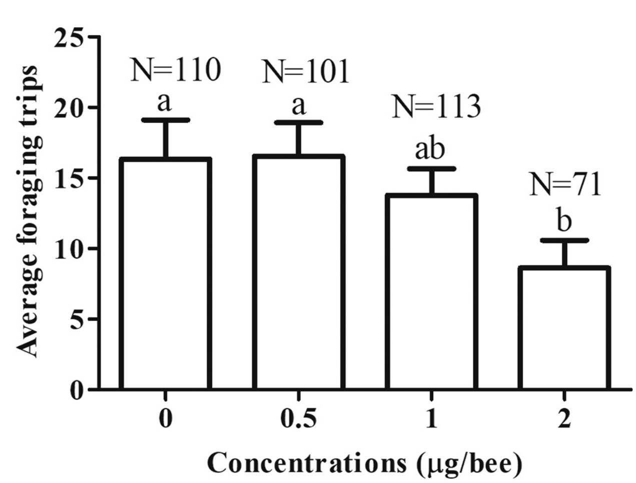

Fig. 3. Acetamiprid decreased the average foraging trips of worker bees *Apis mellifera*. Each group has a single error bar (mean ± *SE*). Different letters above bars indicate significant differences (ANOVA, $P < 0.05$). *N* values denote number of worker bees.

Table 2
Effects of different acetamiprid doses on the average lifespan of *Apis mellifera* worker bees.

Groups (μg/bee)	Average lifespan (days)	Median	Sample size
0.0	17.210 ± 1.014[a]	15	105
0.5	17.560 ± 0.982[a]	15	100
1.0	16.010 ± 1.023[ab]	12	96
2.0	12.861 ± 1.122[b]	8	72

Values (mean ± *SE*) in the same column with different letters are significantly different (Log-rank test, $P < 0.05$).

condition (Hassani et al., 2008), which causes bees to be physically exhausted and appear go-slow phenomenon, then the number of foraging flights of worker bees were affected. The current study provides newly important insights into pesticides that disorder the proper day-off rotation of honey bees, and establish a good foundation for broadly research on rotating day-off status of animals. This study found that the average foraging trips of 2 μg/bee group were also significantly lower than 0 μg/bee group and 0.5 μg/bee group (Fig. 3, Table 1), which was identical to the above results. In addition, it is of importance that not only the pesticide but also its metabolites can be dangerous for honey bees. For example, it has been found that in case of imidacloprid - its metabolite imidacloprid-olefin is more dangerous than the parent compound (Suchail et al., 2001). More recently, the parent compound and its metabolite were found to affect biochemical pathways in bumblebees (Erban et al., 2019b). Therefore, whether acetamiprid or its metabolites pose greater side effects on honey bees need to be further studied. Furthermore, adjuvants of a pesticide are largely assumed to be biologically inert and usually not considered in risk assessments (Stevens, 1993; Hess, 1999). However, recent research has reported that bees are sensitive to adjuvants, like organosilicone surfactants, nonylphenol polyethoxylates and the solvent *N*-methyl-2-pyrrolidone (NMP) (Mullin et al., 2015). For this reason, risk evaluation of the compounds containing in acetamiprid or other pesticides needs to be further studied.

As mentioned above, it is increasingly clear that 2 μg/bee acetamiprid could cause adverse effects on lifespans and foraging behaviors of honey bee workers. Though 2 μg/bee dose of acetamiprid corresponding to the concentration of 1333 mg/L is significantly higher than the recommended field-realistic doses (50–500 mg/L), we still cannot ignore the risk of bees exposed to acetamiprid in practice. Firstly, the dead and poorly state bees in each group were eliminated on the first day after exposure to acetamiprid, and this study aims to assess the effects of chronic exposure to acetamiprid on the lifespans and foraging behaviors of honey bees. While a previous study has shown that over 0.5 μg/bee acetamiprid could pose adverse effects on the learning and memory, and homing abilities of honey bees (Shi et al., 2019a). Secondly, the users subjectively overuse acetamiprid to control pests in the case of severe pests in crops. Thirdly, each bee was dropped 1.5 μL acetamiprid in the current study, while bees may have been exposed to more than this amount during the actual collection process. Moreover, the acetamiprid liquid is also prone to condense under higher temperature or wind weather conditions, indirectly resulting in increased concentration.

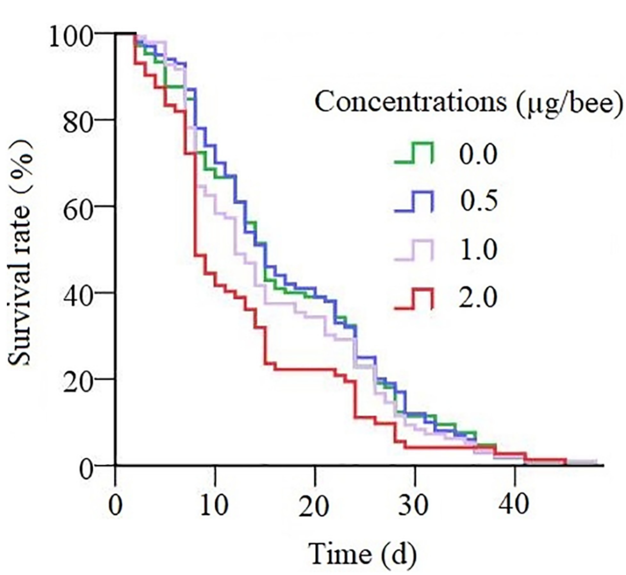

Fig. 4. Acetamiprid reduced the survival of *A. mellifera* workers.

5. Conclusions

Exposure to 2 μg/bee acetamiprid significantly affected the first foraging flights, influenced rotating day-off status, reduced average foraging trips, and shortened lifespans of *A. mellifera* L. worker bees. This work may provide a new perspective into the neonicotinoids that accelerate the colony failure. However, there are several limitations of this study. For instance, the effect of acetamiprid on food collection ability of bees is also worth investigating.

Author contribution statement

In this work, J Shi carried out the laboratory work and wrote the manuscript. X Wu conceived this research and designed experiments. H Yang, L Yu, Y Liu and W Yan contribute to the laboratory work. X Wu, J Shi, C Liao and M Jin performed the experimental analysis and participated in the revisions of this manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Jingliang Shi: Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Heyan Yang:** Investigation, Methodology. **Longtao Yu:** Investigation. **Chunhua Liao:** Methodology, Formal analysis. **Yao Liu:** Investigation. **Mengjie Jin:** Investigation. **Weiyu Yan:** Conceptualization, Resources, Funding acquisition. **Xiao Bo Wu:** Conceptualization, Methodology, Resources, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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