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Honey bee *Apis mellifera* larvae gut microbial and immune, detoxication responses towards flumethrin stress☆

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ABSTRACT

Mites are considered the worst enemy of honey bees, resulting in economic losses in agricultural production. In apiculture, flumethrin is frequently used to control mites. It causes residues of flumethrin in colonies which may threaten honey bees, especially for larvae. Still, the impact of flumethrin-induced dysbiosis on honey bees larval health has not been fully elucidated, and any impact of microbiota for decomposing flumethrin in honey bees is also poorly understood. In this study, 2-day-old larvae were fed with different flumethrin-sucrose solutions (0, 0.5, 5, 50 mg/kg) and the dose increased daily (1.5, 2, 2.5 and 3 μL) until capped, thereafter the expression level of two immune genes (*hymenoptaecin*, *defensin1*) and two detoxication-related genes (*GST*, *catalase*) were measured. Meanwhile, the effect of flumethrin on honey bee larvae (*Apis mellifera)* gut microbes was also explored via 16S rRNA Illumina deep sequencing. We found that flumethrin at 5 mg/kg triggered the over expression of immune-related genes in larvae, while the larval detoxification-related genes were up-regulated when the concentrations reached 50 mg/kg. Moreover, the abundance and diversity of microbes in flumethrin-treated groups (over 0.5 mg/kg) were significantly lower than control group, but it increased with flumethrin concentrations among the flumethrin-treated groups. Our results revealed that microbes served as a barrier in the honey bee gut and were able to protect honey bee larvae to a certain extent, and reduce the stress of flumethrin on honey bee larvae. In addition, as the concentration of flumethrin increases, honey bee larvae activate their immune system then detoxification system to defend against the potential threat of flumethrin. This is the first report on the impact of flumethrin on gut microbiota in honey bees larvae. The findings revealed new fundamental insights regarding immune and detoxification of host-associated microbiota.

1. Introduction

Honey bees (*Apis mellifera*) play an essential role in pollinating crops worldwide (Vanengelsdorp and Meixner, 2010), and the economic benefits of bee pollination were more than 14.6 billion annually in the United States (Elliud et al., 2014). However, the population of bee colonies has a dramatic decline in many countries as a result of mites, diseases, pesticides and inclement weather or some combination of these factors (Tony.,2008). Mites and pesticides residues in colonies were believed to be the main causes (Henry et al., 2012). The mite *Varroa destructor* is parasitic on the body surface of honey bees and suck lymphatic fluid from honey bees, causing honey bees to stunt even death, resulting in serious economic losses in apiculture (Duay et al., 2002; Bowen-Walker and Gunn, 2010). Pyrethroids are commonly used for *Varroa* control (Muhammad et al., 2020), whereas with the widespread use of pyrethroids acaricide, the acaricide residues in honey, propolis, and wax were found in different countries (Christopher et al., 2017). The extensively repeated using of acaricide in colonies causes the increase of mite resistance and reduces the efficacy of the acaricide, and overdose of acaricide would be applied to control mites, which may have stronger adverse effects on honey bee colonies. Flumethrin, which belongs to the pyrethroids group, has been widely used as an acaricide for the control of *Varroa mites* in commercial honey bee keeping throughout the world for many years (Yu et al., 2015). Its commercial name is Bayvarol, plastic Bayvarol strips impregnated with flumethrin (3.6 mg flumethrin per strip) applied to control mites at a dose of 14.4 mg per

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colony with 4 strips (Zhou et al., 2007). Nevertheless, flumethrin induced significant mortality during larval metamorphosis and adult emergence, it was also noteworthy that flumethrin significantly regulated the expression of immune and developmental genes (Qi et al., 2020b), disturbed the physiological and biochemical homeostasis of honey bee workers and larvae *A. mellifera* (Qi et al.,2020a), hurt the lifespan and olfactory learning ability of honey bee workers *Apis cerana* (Tan et al., 2013).

Honey bees develop an immune and detoxication response when they intake flumethrin residual food (Qi et al., 2020b). The gene *GSTS* and *catalase* are essential genes involved in detoxification metabolism in honey bee (Berenbaum and Johnson, 2015; Mao et al., 2011; Boncristiani et al., 2012). The *GSTS* gene family can reduce the damage of oxidative stress (Jia et al., 2014), and *catalase* is one of the protective enzyme systems, which plays an important role in scavenging free radicals and protecting the normal metabolism of insects (Beauchamp and Fridocich,1971; Fridovich, 1978). In addition, *hymenoptaecin* and *defensin1* plays a crucial role in honey bee congenital humoral immune system which belongs to antimicrobial peptide gene family (Casteels et al., 1993), and affect defending pathogens effectively (Aronstein and Saldviar, 2005; Richard et al., 2012). Meanwhile, the microbiota in honey bees also play a crucial role in decomposing toxic chemicals (Wang et al., 2020).

Microbes is highly abundant in honey bees' gut, and the potential role of gut microbial communities in the health of honey bees has recently become more widely appreciated (Engel et al.,2013). The composition of the gut microbiota is different in adult bees and larvae. In addition, microbes are transmitted from adults to newly hatched bees through feeding and secretion inside the colony (Hauke et al., 2013). The gut is colonized with the heterogenic microbiota, which takes part in the digestion and absorption of nutrients, protecting the honey bees against pathogens and parasites, enhancing honey bees' immunity and safeguarding their health (Evans and Lopez, 2004; Hamdi et al., 2011). The disorder of the gut microbiota leads to higher mortality, suggesting the importance of normal gut microbiota in honey bees (Kasie et al., 2017). Currently, studies revealed that some chemicals disturb the composition of the gut community. Antibiotic exposure perturbs gut microbiota, reduces the genetic diversity of core species, and elevates mortality in honey bees (Raymann et al., 2018; Motta et al., 2018). Glyphosate alters the bee gut community and increases susceptibility to infection by opportunistic pathogens (Motta and Moran ,2020; Wang et al., 2021). Additionally, polystyrene microplastics exposure affected honey bees, but gut microbiota can against its risks (Wang et al., 2020).

However, the effects of flumethrin on honey bees has been less reported, especially for larvae, there is no evidence indicating that flumethrin affects honey bees' gut microbiota community, and it is also unclear the interactions between gut microbiota and hosts in the metabolism of flumethrin. Here, we evaluated the effects of flumethrin on the immune genes (*hymenoptaecin*, *defensin1*) and detoxification genes (*GST, catalase*) expression to explore the stress of flumethrin on honeybee larvae. Meanwhile, we also investigated the impact of flumethrin exposure on the diversity and composition of the honeybee larvae gut microbiota, so as to explore honey bee *Apis mellifera* larvae gut-microbial and immune, detoxification responses towards flumethrin stress.

2. Materials and methods

2.1. Chemicals and reagents

Flumethrin (purity: 98.55%, g/g) was purchased from Going Tech Biotechnology Co., Ltd, and the purity of flumethrin is 98.5%. Flumethrin was diluted by 50% (w/v) sucrose solution to concentrations of 0, 0.5, 5 and 50 mg/kg according to the LC50, the residues of flumethrin in period of season and management (Bogdanov et al., 1998; Johnson et al., 2010; Josep and Jose ,2010; Yu et al., 2015; Shyma et al., 2015; Daniele et al., 2018; Jamal et al., 2020). The total RNA of larvae was extracted using an RNA extraction kit (TransZol Up Plus RNA Kit), which was from the Tiangen Biotech Co., Ltd. Reverse transcription kit and RT-qPCR kit (SYBR® Premix Ex Taq™ II) were both purchased from TaKaRa company.

2.2. Honey bee rearing

Three honey bee (*A.mellifera*) colonies were kept in Honey bee Research Institute of Jiangxi Agriculture University, Nanchang city, China (28.46°N, 115.49°E), according to standard beekeeping techniques. All experiments were conducted in a single colony to ensure genetic similarity among the larvae used, and the experiment was repeated three times in three different honey bee colonies. The honey bee colonies were healthy that were not threated by pathogens (foulbrood), parasitic mites, and had no prior exposure to pesticides. We used clean wax to make foundations, then put into honey bee colonies for comb building, and the honey bee queen was controlled and lay eggs in the drawn-out empty comb for 12 h. After that, the eggs were transferred to super-box for hatching into larvae. Five days later, the comb was removed from the hive. The hatched larvae (2-day-old larvae, D2) were divided into four groups and fed with different flumethrin-sucrose solution (0, 0.5, 5 and 50 mg/kg) respectively until capped (6-day-old, D6), each larva from D2 to D5 were fed 1.5, 2, 2.5 and 3 μL flumethrin-sucrose solution, respectively. There were about 300 larvae in each group, and these larvae were reared in the same hive. The 6-day-old larvae were picked from capped cells and cleaned by ultrapure water. We get the larvae samples and each sample contains three larvae. All samples were snap-frozen in liquid N_2 then moved into −80 °C refrigerator.

2.3. Gene expression analysis

The treated larvae on D6 were collected, and three complete larvae were pooled as a sample, each treatment group in a single colony contained six samples. Total RNA was extracted from each sample according to the manufacturer's protocol. The RNA concentration of each sample was measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the purity of the total RNA was determined as the OD260/280 ratio with expected values between 1.8 and 2.0. RNA integrity was determined by running an aliquot on 1% agarose gel. Synthesis of cDNA was performed using Reverse transcription kit, and the reaction was accomplished in T100 Thermal Cycler PCR instrument (Bio-Rad, USA), and the reverse transcripts were preserved in a refrigerator at −80 °C.

Primers were designed with Primer 5.0 software and synthesized at Sangon Biotech (Shanghai, China). The real-time quantitative PCR reaction mixture (10 μL) was as follows: 1 μL of cDNA, 5 μL of SYBR® Premix ExTaq™ II, 0.2 μL of Rox Reference Dye, 0.4 μL each of upstream and downstream primers, and 3 μL of ultrapure sterile water. The real-time quantitative PCR were accomplished on an Applied Biosystems QuantStudio™ 5 real-time PCR instrument. The reaction conditions were as follows: 95 °C for 30 s, 60 °C for 1 min for 40 cycles (Liao et al., 2018). Each reaction had three technical replicates, and the amplification efficiency was between 90 and 110%, *GAPDH* was treated as the internal reference gene, primers information was shown in Table 1.

2.4. Genomics DNA extraction and library construction

Each sample contains three larvae, and each group contains six samples. Larvae with their gut microbiota DNA was extracted by MagPure Stool DNA KF kit B (Magen, China) following the manufacturer's instructions. DNA was quantified with a Qubit Fluorometer by using Qubit dsDNA BR Assay kit (Invitrogen, USA), and the quality was checked by running an aliquot on 1% agarose gel. Variable regions V4 of bacterial 16S rRNA gene was amplified with degenerate PCR primers

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

Gene name	Gene category	Primer sequeces (5′to 3′)
GST	Detoxification	F: TGCATATGCTGGCATTGATT R: TCCTCGCCAAGTATCTTGCT
Catalase	Detoxification	F: GTCTTGGCCCAAACAATCTG R: CATTCTCTAGGCCCACCAAA
Hymenoptaecin	Immune	F: CTCTTCTGTGCCGTTGCATA R: GCGTCTCCTGTCATTCCATT
Defensin1	Immune	F: TGCGCTGCTAACTGTCTCAG R: AATGGCACTTAACCGAAACG
GAPDH	Housekeeping	F: GCTGGTTTCATCGATGGTTT R: ACGATTTCGACCACCGTAAC

(515F: 5′-GTGCCAGCMGCCGCGGTAA-3′, 806R: 5′- GGACTACHVGGGTWTCTA AT-3′).

PCR enrichment was performed in a 50 μL reaction containing 30 ng sample DNA. PCR cycling conditions were as follows: 95 °C for 3 min, 30 cycles of 95 °C for 45 s, 56 °C for 45 s, 72 °C for 45 s and final extension at 72 °C for 10 min. The PCR products were purified using Agencourt AMPure XP beads and eluted in an Elution buffer. Libraries were qualified by the Agilent Technologies 2100 bioanalyzer. The validated libraries were used for sequencing on the Illumina HiSeq 2500 platform (BGI, Shenzhen, China) following the standard pipelines of Illumina and generating 2 × 250 bp paired-end reads.

2.5. OTU cluster analysis and species annotation

Illumina sequence was processed to obtain Clean Data (Douglas et al., 2014), Paired-end reads were merged using FLASH to obtain raw tags (Tanja et al., 2011), and merged tags were clustered into OTUs with UCLUST (Edgar, 2013) (v7 0.0.1090). Then the OTUs were used to assign the taxonomic category by using the SILVA database with RDP classifier (Quast et al., 2013) (v2.2). The OTU representative sequences are obtained, and we compared the OTU representative sequence and the database by RDP Classifier (v2.2), then these OTU sequences were annotated into species, the confidence threshold was set as 0.8. Subsequently, these results were processed: those OTU sequences without annotated results and species not belong to the analysis item were removed.

2.6. Statistical analysis

Statistical analyses were performed in SPSS Statistics version 26, and the abnormal values (values over mean ± 3 times standard error were abnormal) of the gene expression levels and relative abundance among different groups were removed. Alpha indexes were based on the OTUs of each group and calculated using mothur (v1.31.2) (Schloss et al., 2009). All differences among the four groups were determined by one-way ANOVA and Fisher's LSD tests was used to determine if there were any differences among different groups. $P < 0.05$ was considered statistically significant. The larval gene expression level was calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

3. Results

3.1. Larval gene expression analysis

The relative expression of *GST* and *catalase* in 50 mg/kg was the highest, which was significantly higher than the other three groups ($P < 0.05$, one-way ANOVA) (Fig. 1A and B). Also, none of these differences were statistically significant in the relative expression of *GST* and *catalase* among the control group, 0.5 mg/kg and 5 mg/kg group.

The relative expression of *defensin1* and *hymenoptaecin* in 5 mg/kg and 50 mg/kg group were significantly higher than those in control group and 0.5 mg/kg group. The relative expression of *hymenoptaecin* in 50 mg/kg was also significantly higher than 5 mg/kg. However, there was no significant difference ($P > 0.05$) in the relative expression of *defensin1* and *hymenoptaecin* between control group and 0.5 mg/kg group, and *defensin1* between 5 mg/kg and 50 mg/kg group (Fig. 1C and D).

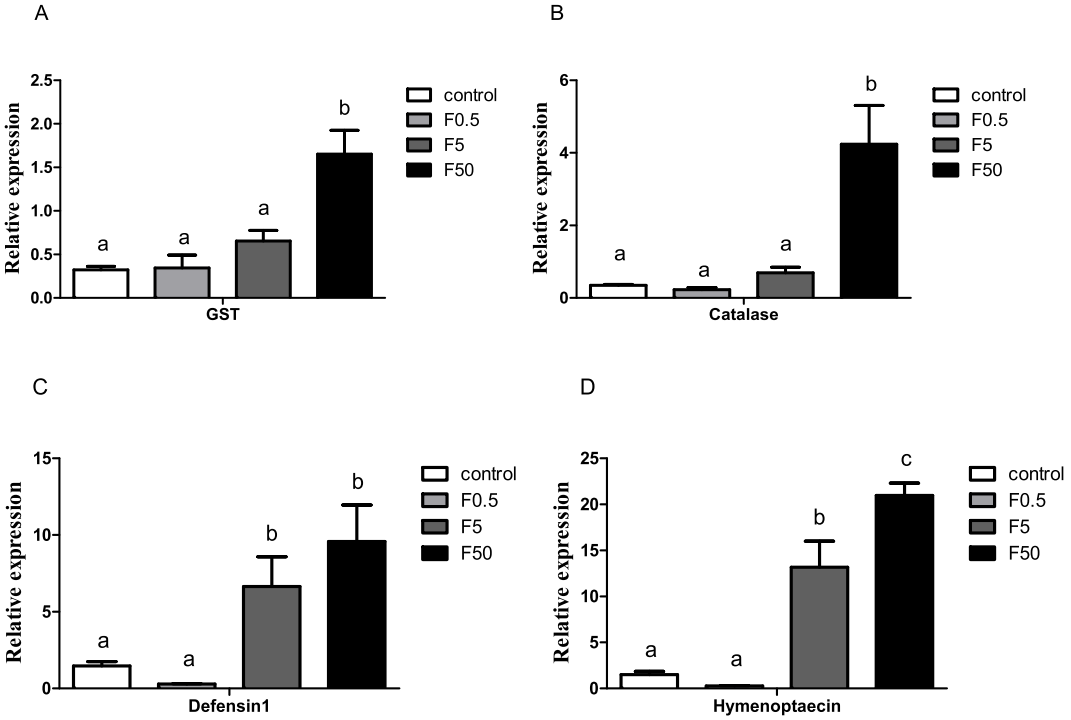

Fig. 1. Expression level of detoxication- and immune-genes in honey bee larvae. Effects of different concentrations of flumethrin on the relative expression levels of larvae. *GAPDH* was used as an internal control gene in each group. Different letters above bars mean significant differences between groups (ANOVA test, $P < 0.05$).

3.2. OTU clustering and noting

Overall, 388 OTUs were commonly shared among control group and 3 flumethrin-treated groups. Additionally, 392 OTUs were specific in control group, 31 OTUs were specific in 0.5 mg/kg group, 195 OTUs were particular in 5 mg/kg group, 189 OTUs were specific in 50 mg/kg group. The maximum number of OTUs in the control group was 1427, followed by 1151 for 50 mg/kg group, 1113 for 5 mg/kg group, and the least for 0.5 mg/kg group was 515. And we compared the obtained OTU representative sequences with the database and annotated these OTU representative sequences with species (Fig. 2).

3.3. Effect of flumethrin on honey bee larvae gut microbiota

The most abundant microbes at the class level in honey bee gut were *Gammaproteobacteria* (52.64%), *Bacilli* (21.69%), *Clostridia* (7.80%), *Alphaproteobacteria* (5.54%), *Betaproteobacteria* (3.65%), *Bacteroidia* (3.04%), *Chloroplast* (2.29%), *Actinobacteria* (0.98%). And the most abundant genus of bacteria in honey bee larvae gut were *Yersinia* (14.73%), *Geobacillus* (9.70%), *Pseudomonas* (7.73%), *Acinetobacter* (4.53%), *Escherichia* (1.72%), *Fructobacillus* (1.16%), *Streptococcus* (0.99%), *Burkholderia* (0.96%). Also, two core bacteria of honey bee were detected: *Lactobacillus, Bifidobacterium.* At the genus level, the relative abundance of *Yersinia*, *Geobacillus*, *Pseudomonas, Acinetobacter*, *Escherichia, Streptococcus, Lactobacillus*, *Bifidobacterium* in the control group was significantly higher than those in three flumethrin-treated groups ($P < 0.05$), and the relative abundance of these bacteria in flumethrin-treated groups increased with increasing concentration (Fig. 3). The relative abundance of *Yersinia*, *Geobacillus*, *pseudomonas*, *Acinetobacter, Escherichia*, *Streptococcus* in 50 mg/kg group was significantly higher than 0.5 mg/kg group. There was significant difference in the relative abundance of *Geobacillus, Pseudomonas, Acinetobacter* among the four groups, the relative abundance in control group was the highest, then in 50 mg/kg group, and the relative abundance in 0.5 mg/kg was the least. However, no significant difference was found in the relative abundance of *Fructobacillus, Burkholderia, Lactobacillus and Bifidobacterium* among the flumethrin-treated groups. We found that flumethrin induced the relative abundance of microbiota declined compared with control group, but the relative abundance of these microbiota gradually increased with the concentration increased.

3.4. Alpha diversity indexes analysis

Six alpha diversity parameters include observed species, Chao, ace, Shannon' s diversity, Simpson' s diversity, Good' s coverage were used to assess the abundance and diversity of samples. As shown in Fig. 4, the observed species, Chao, ace and Shannon' s diversity of 0.5 mg/kg group was significantly lower than the control group. Also, the observed species and Shannon' s diversity of 5 mg/kg and 50 mg/kg group were significantly lower than the control group, but the Chao and ace of 5 mg/kg and 50 mg/kg group were not significantly lower than the control group. Meanwhile, the Simpson' s diversity of control group was significantly lower than the flumethrin-treated groups, and the Good' s coverage of control group was significantly higher than that in 5 mg/kg group.

In addition, the observed species, Chao, ace, Shannon' s diversity of 0.5 mg/kg group was significantly lower than 5 mg/kg group and 50 mg/kg, and the Good' s coverage of 0.5 mg/kg group was significantly higher than 5 mg/kg group, while there was no significant difference in the six alpha diversity parameters between 5 mg/kg group and 50 mg/kg.

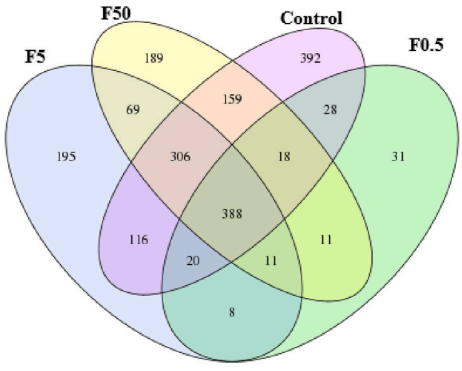

Fig. 2. Numbers of the OTUs in the four groups. Different colors in the graph represent different groups, and the numbers in the two overlapping parts are the number of OTUs shared between two groups. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Flumethrin is used to control *Varroa* mites in beehives, which affects honey bees to a certain extent inevitably, and the agent remained residual easily, which harmed larvae and newly emerged honey bees directly. Many commonly used pesticides including nitenpyram, coumaphos, fluvalinate, and chlorothalonil disturbed gut microbiota and significantly affected the structure of bacterial (Zhu et al., 2020; Kakumanu et al., 2016), it is suspected that the host regulated the expression of related genes and the abundance and composition of gut microbiota to cope with exogenous chemicals.

Immune and detoxication related genes are involved in regulating the immune and detoxification functions of the whole development process of the host, which is essential for survival of the host (Shi et al., 2020). The larval stage is an important part of the bee development process, but the responses of honey bee larva for flumethrin is unclear. In our study, we tested two detoxication-related genes (*GST* and *Catalase)* and two immune-related genes (*Hymenoptaeci*, *defensin1*) expression level of 6-day-old larval treated with different concentrations of flumethrin. Our results showed that flumethrin had an up-regulation on expression of the detoxification-related genes of honey bee larvae at a high concentration (50 mg/kg), while it upregulates the expression of immune-related genes of honey bee larvae at a low concentration (5 mg/kg). The immune and detoxication genes of larvae were not activated in 0.5 mg/kg, it was possible that the toxicity of flumethrin was eliminated by these microbes (Kwong et al., 2017; Danihlík et al.,2015). There was special mechanism exists for larvae to resist the threat of low concentrations of flumethrin toxicity (Wang et al., 2020), and consequently the balance of biochemistry and physiology was not disturbed by the low concentration of flumethrin. However, the immune-related and detoxication-related genes were activated in 5 mg/kg group and 50 mg group, probably because a series of immune responses and detoxifying abilities were then initiated to against the stress for a higher concentration of flumethrin.

Moreover, the effect of flumethrin on larval gut microbiota were further explored. Similar to other reports (Kakumanu et al., 2016; Wang et al., 2020), the dominant bacteria at class level in our results were *Gammaproteobacteria, Alphaproteobacteria, Bacilli, Actinobacteria, Bacteroidia, Betaproteobacteria, Chloroplast, Clostridia*. And the most abundant genus in larvae were *Yersinia*, *Geobacillus*, *pseudomonas*, *Acinetobacter*, *Escherichia, Fructobacillus*, *Streptococcus*, *Burkholderia.* The dominant composition of microbiota in honey bees is intensely involved with its environment (Zhao et al., 2018). Besides, each bacterium has its function and the bacteria is benefit for their hosts. The composition of the microbiota of larvae was highly similar to adult bees (Kakumanu et al., 2016; Wang et al., 2020), but the numbers of bacteria communities were less than adult bees. Vast changes were observed in the diversity of gut microbiota among the four groups. It is apparent that the number of OTUs in the control group was the highest compared with the other three flumethrin-treated groups, but it was gradually increased

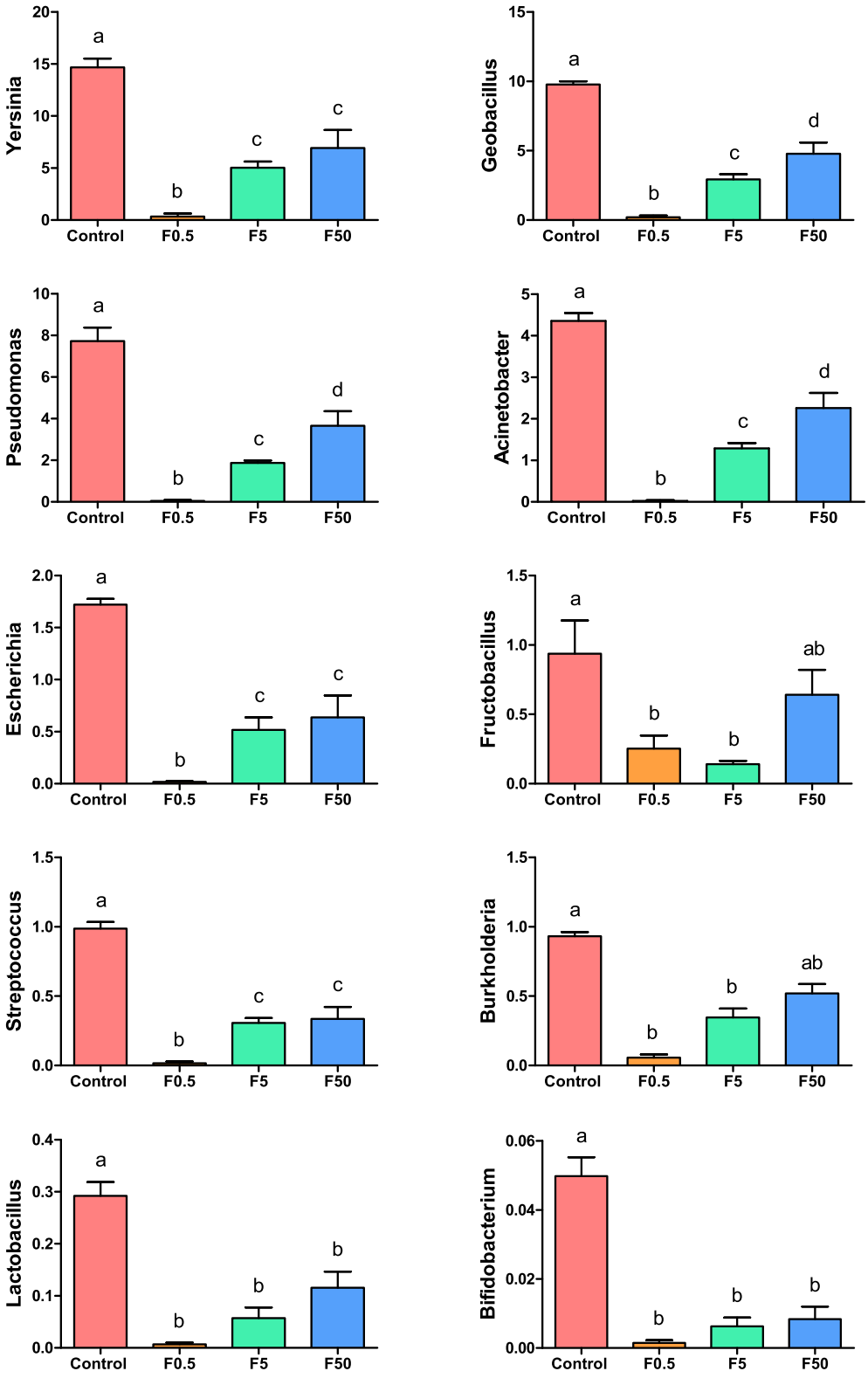

Fig. 3. Effect of flumethrin on honey bee larvae gut microbiota. Differences in the dominant bacteria level among the control and flumethrin-treated groups. Different letters above bars mean significant differences between groups (ANOVA test, $P < 0.05$).

with the concentration of flumethrin increasing. Meanwhile, the Good' s coverage index of all groups was more than 99.7%, indicating that at least 99.7% of microbiotas were detected. Also, alpha diversity indexes and the numbers of OTUs showed that the diversity and relative abundance of microbes in the gut were decreased when honey bee larvae consumed different concentrations of flumethrin. However, the diversity and abundance of larval gut microbiota in flumethrin-treated groups were increased with the concentration of flumethrin increasing, which is contradicted with the increasing toxicity of flumethrin. It may be that the effect of flumethrin on honey bee larval gut was mitigated by a specific mechanism. Intestinal microorganisms and immune and detoxification gene expression of honey bee resist the threat of pesticides

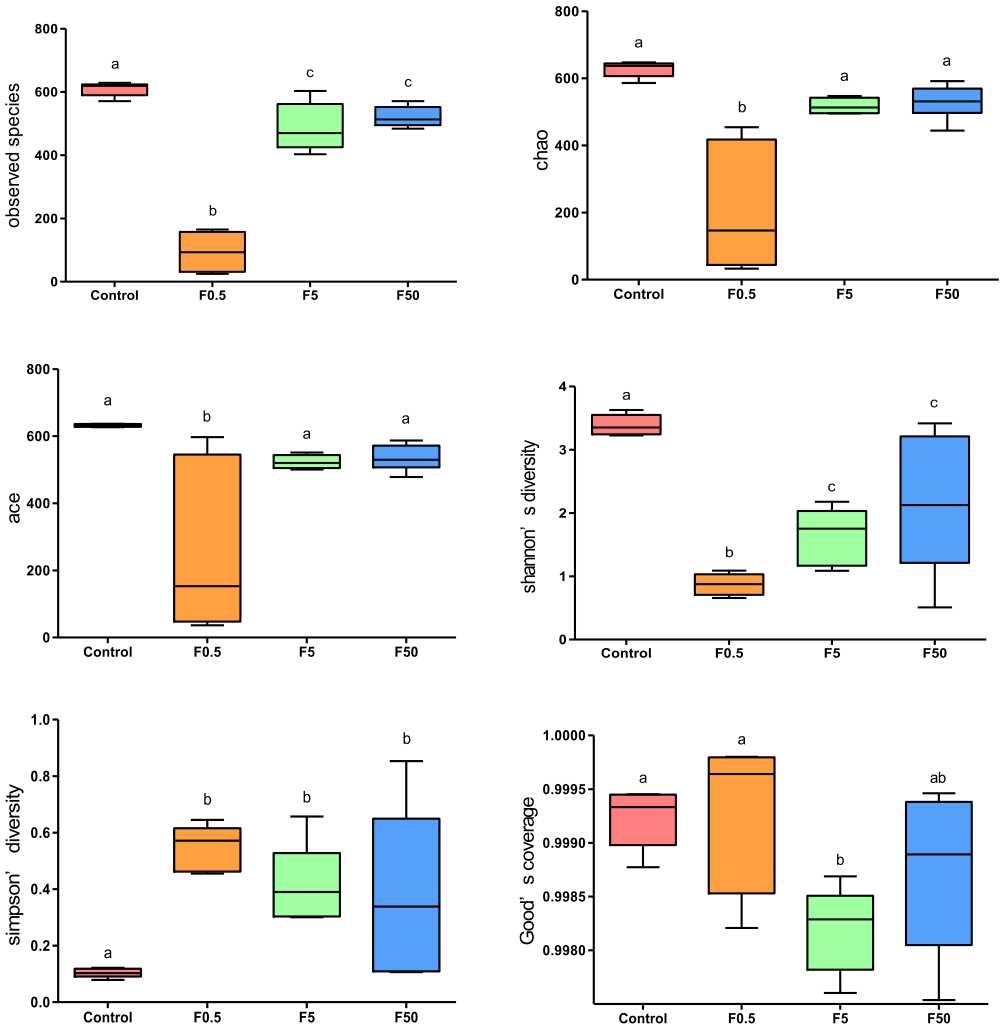

Fig. 4. Boxplot of alpha diversity. The observed species, Chao, ace show the OTU abundance, and Shannon's diversity and Simpson's diversity show the OTU diversity, while Good's coverage shows the coverage of all samples' library. Boxplots indicate the medians (central horizontal lines), interquartile ranges (boxes), and 95% confidence intervals (whiskers). Different letters above boxplots mean significant difference between groups (ANOVA test, $P < 0.05$).

together.

Many studies have reported recently that the gut microbiota promoted the immunity and detoxication of the host to defend against toxic chemicals (Wang et al., 2020; Kamada et al., 2013; Liu et al., 2013). Additionally, the gut microbiota protects the larval intestine against exogenous chemicals, which can participate in metabolizing pesticide and even replace the insects' own metabolic mechanisms (Ramya et al., 2016). Also, the gut microbiota upregulated the expression of host detoxification enzyme genes to respond to pesticide stress and gut microbes play an even greater role in pesticide resistance than the hosts (Li et al., 2011). However, it is uncertain whether microbiota in honey bee gut degrading flumethrin, which is able to reduce the risk of flumethrin for host. In the present study, the abundance and diversity of bacteria communities in the larval gut increased with increasing concentrations of flumethrin exposure, indicating that the microbial threat from flumethrin was decreasing, but the exact mechanism for this phenomenon occurs is not well understood. We guessed that this phenomenon maybe resulted by microbiota-host interactions. At low concentration (0.5 mg/kg), the expression levels of immune and detoxification-related genes were not affected, while profound changes have taken place in gut microbes, suggesting that the metabolic and resistance of flumethrin is dominated by microbes, or gut microbes are more susceptible to flumethrin. Definitely, this stabilization of the host's physiological state was at the expense of gut microbes. Nevertheless, at a higher concentration (50 mg/kg), significant differences among the four groups were found, and the diversity and abundance of microbiotas in 50 mg/kg group were the highest. Highly expressed larval detoxification genes may be associated with the abundance of gut microbes (Liu et al., 2013), and flumethrin can affect the larval detoxification system in a quite higher concentration, or larval detoxification system was participated in defending against flumethrin. When the concentration of flumethrin beyond the tolerance of microbiotas, the microbiotas activate the host's immune system through a series of mechanisms, and the host cooperate with its gut microbes to defend against the threat of flumethrin. Based on the results, we can conclude that the microbiota is barrier for honey bee which mitigate the risk of flumethrin to some extent, and expands the host's tolerance to the environment. However, our results are too coarse to represent changes in the overall immune and detoxification system of honey bee larvae, and it is unclear which microbiota are making the major contribution to the degradation of flumethrin. The role of gut microbial-host interactions in the metabolism of flumethrin and its specific mechanisms need to be further investigated.

5. Conclusion

Our results revealed that flumethrin reduced the relative abundance

and diversity of gut microbes in larvae. But the relative abundance and diversity of gut microbes were increased while and the immune and detoxification genes were activated gradually with increasing concentrations of flumethrin. The mechanism of intestinal microorganisms and host resistance to pesticide stress needs to be further studied.

Author contribution statement

In this work, X Wu conceived this research and designed the experiments. L Yu carried out the laboratory work and wrote the manuscript. H Yang, F Chen, Z Wu, Q Huang, X He, W Yan and L Zhang contribute to the laboratory work. All authors read and approved the final manuscript.

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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