

## Neurotoxic pesticides induced alterations in nursing behavior and foraging-related genes in the nurse bee of the honey bee, *Apis mellifera*. L

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### Student Poster

#### Abstract

Honey bee, a vital pollinator in the natural and agricultural ecosystem, has been experiencing a global population decline, mainly attributed to pesticides. Both forager bees and nurse bees are exposed to pesticides, and the main exposure route in nurse bees is pesticide-contaminated food sources. Previous studies showed neurotoxic pesticides triggered an abnormally early transition of nurses to forager bee-like states, but their effects of pesticides on nursing behavior remain unclear. Therefore, we investigated the effects of five neurotoxic pesticides (acetamiprid, imidacloprid, carbofuran, cypermethrin, and fipronil) on nursing behavior in this study. Considering the possible exposure routes in nurse bees, nurse bees were exposed to field-realistic concentrations of pesticide-laced sucrose solution *ad libitum* for 10 days. After chronic exposure to pesticides, nursing behavior was assessed in three aspects: 1) the duration of nursing behavior per visit to the queen cell; 2) the total duration of nursing behavior; 3) the number of bees visiting to a queen cell. Moreover, we analyzed the transcriptome profiles of genes such as *cGMP-mediated kinase protein foraging* (*For*), *Kruppel homolog 1* (*Kr-h1*), *Adenylate cyclase 3* (*Ac3*), *Probable nuclear hormone receptor HR 38* (*Hr 38*), and *Early growth response 1* (*Egr1*), which are related to foraging activity and division of labor to understand the physiological mechanism underlying changes in caste differentiation and behavior using qRT-PCR. As a result, pesticides generally reduced all observed aspects of nursing behavior. In reverse, all selected foraging-related genes were upregulated by the treatment of pesticides, suggesting the inversely proportional relationship between the expression of tested genes and nursing behavior. These findings imply that pesticide-induced disruption of nursing behavior may alter colony composition, potentially leading to colony collapse. This study highlights the sub-lethal and chronic risks of neurotoxic pesticides, even at field-realistic concentrations, emphasizing the need for cautious pesticide use to prevent honey bee population declines.

**Keywords:** Honey bee, Nurse bee, Neurotoxic pesticides, Nursing behavior, Foraging-related genes

#### Introduction

##### ❖ Pesticides and Honey Bee

- **Pesticide**
  - Indispensable with modern agriculture to prevent pests
  - A primary factor contributing to **honey bee population decline**
- **Effect of pesticide exposure on honey bee caste differentiation**
  - Causes **abnormal precocious transition** from nurse bee to forager bee (e.g., acetamiprid, imidacloprid)

→ However, it is unknown how this precocious transition affects brood-rearing behavior, the primary task of nurse bees.

#### Results & Discussion

#### Materials & Methods

##### ❖ Pesticide and Pesticide Treatment

- **Concentration:** Arithmetic "field-realistic" concentration, determined by averaging the mean residue concentrations in bee bread and honey samples collected in previous studies.

Table 1. Pesticides used in this study and their concentration

| Pesticide Group | Pesticide Name     | Concentration (ppb) |
|-----------------|--------------------|---------------------|
| Neonicotinoid   | Acetamiprid (ACE)  | 11.05 ± 6.16        |
|                 | Imidacloprid (IMI) | 4.58 ± 0.97         |
| Carbamate       | Carbofuran (CAB)   | 8.15 ± 2.19         |
| Pyrethroid      | Cypermethrin (CYP) | 42.05 ± 5.63        |
| Phenyl Pyrazole | Fipronil (FIP)     | 2.37 ± 0.16         |

- Newly emerged bee are chronically exposed to pesticides for 10 days.

##### ❖ Nursing Behavior Assay

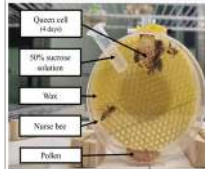


Fig 1. Experimental setup for nursing behavior assay

- Conducted after 10 days of pesticide exposure, using a queen cell

##### ■ Behavioral index

- 1) Average number of bees performing nursing behavior (NI)
- 2) Average number of visits to the queen cell (NV)
- 3) Average total duration of nursing behavior (TD)
- 4) Average duration of nursing behavior per visit to queen cell (DV)

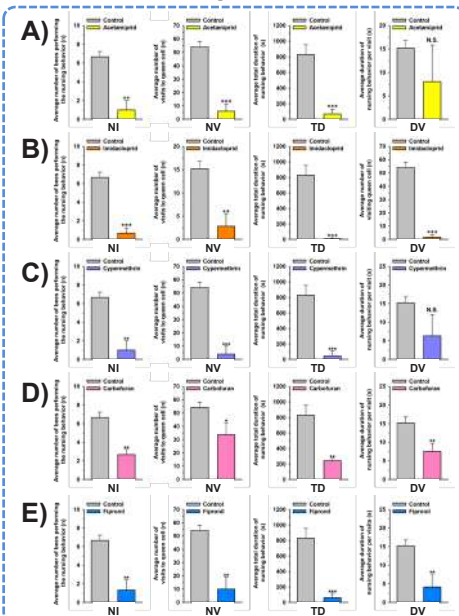
##### ❖ Transcriptome Analysis of Foraging-associated Genes Using qRT-PCR

- Total RNA extraction from pooled three heads → cDNA synthesis and qPCR

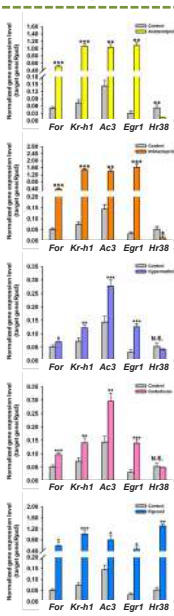
Table 2. Gene information used in this study

| Gene function            | Gene Name                                     | Gene symbol  |
|--------------------------|-----------------------------------------------|--------------|
| Foraging-associated gene | <i>cGMP-dependent protein kinase foraging</i> | <i>For</i>   |
|                          | <i>Kruppel homolog 1</i>                      | <i>Kr-h1</i> |
|                          | <i>Adenylate cyclase 3</i>                    | <i>Ac3</i>   |
|                          | <i>Early growth response protein 1</i>        | <i>Egr1</i>  |
|                          | <i>Probable nuclear hormone receptor 38</i>   | <i>Hr38</i>  |
| Reference gene           | <i>40s ribosomal protein S5</i>               | <i>RPS5</i>  |

#### Nursing Behavior



#### Gene Expression



##### ❖ Relationship between Nursing Behavior and Foraging-related Genes

- **Inverse relationship following pesticide exposure**
  - Nursing behavior **decreased**.
  - Gene expression levels **increased**.

##### ❖ Even Field-realistic Concentrations Can Significantly Affect Nursing Behavior and Foraging-related Genes

- **Concentrations used in this study**
  - Based on the **field-detected concentration**

→ However, even trace amounts of neurotoxic pesticides noticeably **decreased nursing behavior** and **increased the expression of foraging-related genes**.

##### ❖ Possibility of Precocious Caste Differentiation Induced by Neurotoxic Pesticides

- **IMI and ACE**
  - Previously reported as **causes of precocious foraging**.
- **Other neurotoxic pesticides**
  - Showed patterns similar to ACE and IMI in both behavioral indices and gene expression.

→ These neurotoxic pesticides may also **trigger precocious caste transitions** in nurse bees.

##### ❖ Potential Risk of Colony Collapse due to Abnormal Early Caste Transition

- **Imbalance** between nurses and foragers

→ This **imbalance** could ultimately lead to **colony collapse**.

Fig. 2 Effect of neurotoxic pesticides on the nursing behavior of nurse bees and on the expression of foraging-associated genes. A) Acetamiprid; B) Imidacloprid; C) Cypermethrin; D) Carbofuran; E) Fipronil. Statistical analysis was conducted using Student's t-test to compare differences between the control group and the treatment group (N.S.:  $p > 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

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