



The sublethal effects of ethiprole on the development, defense mechanisms, and immune pathways of honeybees (*Apis mellifera* L.)

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Abstract Ethiprole has been widely used in agriculture, but there have been few studies on the adverse effects of ethiprole on nontarget organisms. This study focused on the mechanism of the sublethal effects of ethiprole on the development, antioxidation mechanisms, detoxification mechanisms and immune-related gene expression of honeybees (*Apis mellifera* L.). Honeybee larvae were found to be more sensitive than pupae to ethiprole. It was found that ethiprole inhibited the pupation and eclosion of bee larvae in a dose-dependent manner, with ethiprole doses of 1×10^{-5} mg/L decreasing pupation and eclosion rates to $50.00 \pm 8.84\%$ and $20.83 \pm 10.62\%$,

respectively. The activities of antioxidative enzymes (superoxide dismutase and catalase) and detoxification factors (glutathione and glutathione S-transferase) were also significantly increased in ethiprole-exposed honeybees, indicating that a sublethal dose of ethiprole also induced oxidative stress in honeybees. In the 1×10^{-5} mg/L ethiprole-exposure group, the expression of pathogen recognition-related gene *PGRP-4300* was upregulated 11.10 ± 0.45 -fold, and that of detoxification-related gene *CYP4G11* was upregulated 8.84 ± 0.11 -fold, indicating that ethiprole induced an immune reaction in honeybees. To the best of our knowledge, this study represents the first demonstration that sublethal concentrations of ethiprole inhibit honeybee development and activate honeybee defense and immune systems.

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Introduction

Honeybees are responsible for most of insect pollination of agricultural ecosystems (A. M. Klein et al. 2007), and thus play an essential role in ecosystem functioning and agricultural production (A. Klein et al. 2003). In this context, it is notable and concerning that instances of honeybee colony collapse disorder (CCD) have been frequently reported in the United States and

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