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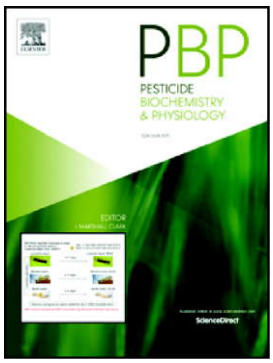

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Acute toxicity and sublethal effects of myclobutanil on respiration, flight and detoxification enzymes in *Apis cerana cerana*

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Abstract

Myclobutanil is currently used on the flowering plants. Little is known about how *Apis cerana cerana* respond to myclobutanil exposure. Hence, the acute toxicity of myclobutanil and its sublethal effects on respiration, flight and detoxification enzymes [7-ethoxycoumarin O-deethylase (ECOD) and glutathione S-transferases (GSTs)] in *A. cerana cerana* were investigated. The results indicated that formulation grade myclobutanil showed moderate toxicity to *A. cerana cerana* either contact (LD_{50} = 4.697 μg / bee) or oral (LD_{50} = 2.154 μg / bee) exposure. Sublethal dose of myclobutanil significantly reduced the respiration rate of workers at 24 h and 48 h regardless of the exposure method. However, myclobutanil didn't significantly affect the take-off flight. After nurse bees exposure to the dose (LD_5) of formulation-grade myclobutanil, ECOD activity was significantly induced when compared with control, but GST activity didn't change. In the forager bees, no enzyme markers response was obtained in this test. From the present study we can infer that myclobutanil disturb respiration and P450-mediated detoxification of the individual bees of *A. cerana cerana*. Thus, myclobutanil may has risk for *A. cerana cerana*, it should be cautiously used.

Keywords: *Apis cerana cerana* Respiration Flight Detoxification enzymes Myclobutanil

1. Introduction

The reduction of species and population number in managed and wild pollinators has been drawing continuous attention worldwide. However, the causes of such declines remains controversial [1]. The prevailing general consensus goes to a complex of interactions, among which, stressors (complexes), pesticides, fungicides, parasites and habitat loss are believed to be the front line set of culprits [1, 2].

Fungicides are perhaps the most common chemicals that honey bees encounter, because these are sprayed onto blooming flower of crop that are highly attractive to foraging bees [3]. Many honeybee colonies are deliberately transported by commercial beekeepers into areas where different crops and orchards are grown to coincide with blooming times [4]. Just because of this, pollen stores and wax combs of honey bees are often contaminated with fungicides [5]. Although fungicides are generally considered as relatively safe to bees, their active compounds have been revealed, in certain conditions, to produce harmful effects [6]. For instance, orally administered propiconazole showes delayed and acute toxicity to *Osmia lignaria* Say and *Apis mellifera* [7]. Larvae of *A. mellifera* reared on a diet containing 34 mg/L chlorothalonil suffer up to 60% mortality [8]. Boscalid and pyraclostrobin have also been confirmed to affect the nutrition and immune function of bee colony, including pollen consumption, protein digestion, hemolymph protein titers, and changes in viral levels [9]. Besides, synergistic effects are observed when honeybees exposed to ergosterol biosynthesis inhibitor (EBI) fungicides and some pyrethroids or neonicotinoids [10, 11].

Triazole fungicides belong to the broad group of EBI, as major class of fungicides, are widely used in agriculture worldwide. Unlike insecticides that often targeted neural functions, trizaoles inhibit the synthesis of ergosterol, and thus affect

the function of fungal cell membranes in pathogens [12]. Myclobutanil is a important systemic trizaole fungicides, it was developed by Dow Agro-Sciences and first marketed in 1989 [13]. Currently, myclobutanil is widely used to control various fungal diseases in fruits, cereals, vegetable crops, and also in lawn care and wood preservations. It is formulated as a wettable powder, emulsifiable concentrate, granular, dust, dry flowable and ready to use forms. It is also known to be stable to both hydrolysis and photolysis. As a treatment to crops, this compound is applied at multiple plant growth stages (e.g. seed, pre-bloom, bloom, foliar, post-bloom etc).

The adverse effects of myclobutanil on non-target species cannot be ignored. Myclobutanil demonstrates moderate acute toxicity to birds [14] and *A. mellifera* honeybees [15]. It has been confirmed that myclobutanil can elevate the apoptotic cell death in the midgut, salivary glands and ovaries of developing *A. mellifera* larvae [16]. Its residue toxicity has been found to affect the reproductive abilities of test animals and to cause various degrees of hepatic toxicity and disrupts steroid hormone homeostasis in rodents [13].

As a pollinator of flowering plants, the Chinese honeybee, *A. cerana cerana* is a valuable indigenous species that plays an indispensable role in the balance of regional ecology and agricultural economic development [17]. In recent years, farming this species has become extremely difficult. To our knowledge, bee losses as massive death of *A. cerana cerana* foragers has been observed by farmer and my laboratory staff after the use of trizaole fungicides in greenhouses where even insecticides were limited to be used on crops during blooming [18]. Thus, further analysis and determination the potential side effect of fungicide on *A. cerana cerana* is essential and urgent research agenda. Nevertheless, few datas concerning the assessment of myclobutanil toxic effects in *A. cerana cerana*, which we believe has to be maximized

for better understandings and actions.

In this study, we focused on determining acute toxicity and the sublethal effects of myclobutanil on respiration, flight and detoxification enzymes in *A. cerana cerana* honeybees. Respiratory mechanisms in honeybees are mainly mediated by coordinated motor activity [19]. The flight of bees is the most energy-demanding activity. Despite oxygen uptake representing the summation of the energy requirements for the insect physiological processes [20], there are only a few studies that use respiration rate (measured as O_2 uptake or CO_2 release) to demonstrate the possible physiological effects of exposure to insecticides [21]. Moreover, analysis of enzymatic markers can provide information on toxicant effects at sublethal level [22]. EBI fungicides can affect non-target organisms by interfering with a broad range of cytochrome P450 monooxygenases [23]. So the enzymatic markers selected for this study include cytochrome P450 enzymes which is involved in the oxidative metabolism of a plethora of endogenous and exogenous substrates [24] and glutathione S-transferase, a family of enzymes with a key role in the general biotransformation of xenobiotics and endogenous substances [25]. In addition, a comparison between toxicities of formulation (12.5 % emulsifiable concentrate) and technical-grade pesticide to forager will be done. It has been reported that commercial formulations often exhibit higher toxicity to other non-target organisms than the corresponding active ingredient [26-28]. Hence, this kind of information is necessary and crucial for risk assessment on *A. cerana cerana* honeybees.

2. Materials and methods

2.1. Honey bees

The honey bees used in this study were from *Apis cerana cerana* colonies maintained in the apiary of the Chinese Academy of Tropical Agricultural Sciences,

Haikou (N19º32´, E109º32´). Experiments were conducted in the Laboratory of Environment and Plant Protection Institute. Colonies and units did not present visible symptom of any known diseases, and no hives were treated with pesticides.

2.2. Chemicals

Formulation grade myclobutanil (12.5 % EC) was purchased from Shenyang Kechuang Chemicals Co., Ltd. Technical-grade myclobutanil (purity, 96.2 %) was supplied by Hainan Bosswell Agrichemical Co., Ltd. 1-chloro-2,4-dinitrobezene (CDNB), $NADPHNa_4$, 7-ethoxycoumarin (7-EC), 7-hydroxycoumarin (7-HC), sodium dodecyl sulfate (SDS) were products of Sigma Chemical Co. (St. Louis, MO). Reduced glutathione (GSH), phenylmethylsulfonyl (PMSF), dithiothreitol (DTT), Coomassie brilliant blue G250 and bovine serum albumin (BSA) were obtained from Solarbio Science & Technology Co., Ltd., Beijing. The other chemicals were of analytical quality and purchased from commercial suppliers.

2.3. Dose-mortality bioassays

The acute toxicity of myclobutanil to foraging bees was evaluated by two methods, oral administration through spiked syrup and topical application at controlled laboratory conditions. On the day of experiment, usually at 8 o'clock in the morning, bee foragers were gathered from the hive entrance using plastic cups (diameter 6.5 cm, height 16 cm) with approximately 20 individuals, which were subsequently transported to the laboratory.

For the oral administration assay, five concentrations of 81.92, 102.4, 128, 160, 200 mg / L were prepared in 50 % (w / v) aqueous sucrose solution. Before treatment, the bees were starved for 2 h. Then they were anesthetized by cooling (4 °C for no longer than 3 min). Ten bees were counted into the iron cage (13 cm × 6 cm × 10 cm) and provided with 150 μL of myclobutanil-contaminated sucrose solution or only

sucrose solution as control in plastic feeders produced by eppendorf tube. These feeders were weighed before and after filling treated diet. After 4 h (± 30 min), the feeders were removed from the cage and weighed, and replaced with new feeders containing 50 % sucrose solution alone. The sucrose solutions are then provided ad libitum. The dose consumed was determined by comparison of the weight of the dose remaining in the feeders with the weight of a known volume of the test solutions.

For the topical application technique, the honey bees were mildly anaesthetized by cooling (the same as above) and 1 μL of myclobutanil solution in acetone, containing the appropriate dose (2.0, 4.0, 6.0, 8.0, 10.0 μg a.i. / bee), was applied to the dorsal thorax by means of a micropipette. Control bees were treated with the same volume of acetone (≥ 99.9 %). Ten bees were placed in the cage (the same as above) and were provided with 50 % w/v aqueous sucrose solution ad libitum.

All tests were repeated three times and performed at 28 ± 1 ℃, 65 ± 5 % relative humidity in the dark except during observations. Mortality was assessed after 48 h. The bees were considered dead if they were unable to respond when prodded with a fine hair brush. The LD_{50}, LD_5 values for myclobutanil were estimated by probit analysis.

According to the GB/T 31270.10—2014 guideline (PRC National Standard) [29], the toxicities to honeybee are categorized into four grades: (a) $LD_{50} \leqslant 0.001$ μg a.i. / bee, hypertoxicity; (b) 0.001 μg a.i. / bee $< LD_{50} \leqslant 2.0$ μg a.i. / bee, high toxicity; (c) 2.0 μg a.i. / bee $< LD_{50} \leqslant 11.0$ μg a.i. / bee, moderate toxicity; (d) $LD_{50} > 11.0$ μg a.i. / bee, low toxicity.

2.4. Respiration rate and flight bioassays

Respiration rates were estimated from CO_2 production rates using methods slightly modified as described by Tomé et al [21]. CO_2 production was measured

using Qubit systems (Q-Box RP1LP Low Range Respiration Package, Canada). The forager bees were exposed or not exposed to myclobutanil solutions (oral administration LD_5: 1.085 µg a.i. / bee and contact dose LD_5: 1.790 µg a.i. / bee) for 3 h or 24 h or 48 h as described previously before CO_2 production were measured. Three replicates were used for each treatment and each replicate included six bees. Each bee from treatment or control cage was transferred within 1 min to 25 mL glass chambers connected to an open flow system. The measurements were obtained by injecting CO_2-free air (CO_2 scrubbing column with soda lime (white Q13025) absorbed CO_2 from the air) into the chambers for 2 min at 300 mL min^{-1} flow. Air from the chamber passed through a blue DRIERITE drying column and then to a Q-S151 CO_2 analyzer. The air current directed the bee-produced CO_2 to an infrared reader connected to the system allowing the prompt quantification of CO_2 (mL) produced per hour.

Forager bees coming from the same treatment groups of respirometry bioassays were conducted on flight bioassays 24 h after the period of exposure according to Tomé's method [21]. A 120 cm tower was formed with three layer wooden cages covered with iron wire gauze at four sides (50 cm each) and opened in their interior to allow free insect flight through them. A fluorescent lamp was placed the top of the tower in a dark room. Ten forager bees were released in the center bottom of the tower, which were maintained for 1 min and number of bees taking-off for flight were recorded. The flight activity was designed as follows: (a) no flight (i.e. bee remained on the base of the tower), (b) flight up to 35 cm high, (c) flight between 36 and 70 cm high, (d) flight between 71 and 105 cm high and (e) flight reaching the light source at a height of 120 cm high. Three replicates were used for each treatment and control.

2.5. Biochemistry assays

2.5.1. Exposure to insecticide and tissue extracts

Adult foragers collected from hive and 3 days old worker bees were selected for the test. Frames of sealed brood were taken from hives and transferred to a dark humid incubator at 34 ℃ so that bees would eclose. Newly eclosed adults were brushed from frames every 24 h and kept as groups of approximately 30 workers in iron cage (the same as above) and offered 50 % w/v aqueous sucrose solution ad libitum. Nurse bees and forager bees were treated with of myclobutanil solutions at contact dose of LD_5 (1.790 μg / bee) and LD_{50} (4.697 μg / bee) (as described above), which made from formulation-grade and technical-grade respectively. Groups of at least ten bees were exposed per treatment. Three replicates were done for each treatment and control. Test lasted 48 h. At the end of the test, the living bees were anesthetized by cooling at 4 °C for 3 min and sacrificed in ice. For each tissue extract, five midguts were obtained by pulling the sting from honey bees and placed in 1.5 mL centrifuge tubes, flash frozen in liquid N_2, and used for P450 and GST determinations, respectively. Samples were stored at -80℃until enzymatic analysis.

2.5.2. Cytochrome P450 monooxygenase activity assay

Monooxygeanse from midguts of bees was prepared by the method of Lee and Scott [30]. The tissues were homogenized using a MY-10 tissue grinder (Germany). Then add ice-cold extraction solution (0.1 M sodium phosphate buffer, pH 7.5, containing 15 % glycerol, 1.0 mM EDTA, 0.1 mM DTT and 1.0 mM PMSF). The homogenate centrifuged at 10,000 g for 20 min at 4 °C and the supernatants were transferred to new tubes to serve as the enzyme sources.

The ECOD activity was determined using 7-EC as a substrate and the method of Aitio [31] as modified by Li et al [32]. The 1.0 mL reaction mixture containing Tris–HCl buffer (0.25 M, pH 8.0), NADPH (0.25 mM), BSA (0.4 mg/ml),

7-ethoxycoumarin (0.5 mM) and the crude enzyme preparation was added to each test tube. The reaction was started by adding the crude enzyme. The test tubes were incubated for 15 min at 37 ℃ while shaking at 220 rpm using a shaking table (Guowang ZD-85, China).The reaction was stopped by the addition of 0.3 ml of trichloroacetic acid (5 %) and the reaction mixtures were centrifuged at 4 ℃, 7000 g for 3 min. The supernatant was transferred to new test tube and added 0.7 ml Gly-NaOH (0.6 M, pH 10.4). Fluorimetric detection was done with an excitation wavelength of 368 nm and an emission wavelength of 456 nm with Fluorescence Spectrometer (Shimadzu RF-5301PC, Japan).

2.5.3. GST activity assay

The GST activity was measured as described by the method of Habig et al [33] and modified by Li et al [32]. The frozen tissue samples were homogenized in ice-cold buffer (0.1 M phosphate buffer, pH 6.5, containing 1.0 mM EDTA and 1.0 mM DTT). After centrifugation (4 ℃, 10000 g, 30 mim), the supernatant was used to determine GST activity. GST was measured by mixing of 800 μL of 0.1 M phosphate buffer (pH 6.5) containing 30 μL of 30 mM GSH, 40 μL of enzyme extract, 30 μL of 30 mM CDNB as a substrate. The reaction was started by adding CDNB. The change in absorbance was immediately recorded at 340 nm during the initial 2 min using a UV spectrophotometer (Shimadzu UV-2600, Japan). The amount of glutathione conjugate formed was calculated using an extinction coefficient of 9.6 mM^{-1} cm^{-1} for CDNB.

2.5.4. Protein assay

Protein concentration in the samples was determined by the Bradford method [34], at 595 nm, using bovine serum albumin as standard.

2.6. Statistical analysis

The dose-mortality bioassay data was subjected to probit analyses to estimate the toxicological parameters LD_5 and LD_{50}.The data obtained from respirometry bioassay was subjected to Independent-Samples Kruskal-Wallis test and the difference in the same time was adopted one way analysis of variance (ANOVA) after being checked for normality and homoscedasticity, which were satisfied. The results of flight take-off were subjected to the (non-parametric) Kruskal-Wallis test. All biochemical assays data were analyzed by one way analysis of variance (ANOVA) and the means were compared by a Tukey's HSD test. All statistical tests were performed using the IBM SPSS statistics version 19.0 software program (Statistical Package for Social Science, USA). P-values below 0.05 were considered significant.

3. Results

3.1. Acute toxicity

The results of toxicity bioassays of formulation and technical grade myclobutanil to forager bees were summarized in Table1. Mortality in all control groups was always below 5 %. For the topical method, the LD_{50} Values of formulation and technical grade were 4.697 μg / bee and 21.586 μg / bee, respectively. For the oral administration, the LD_{50} Values of formulation and technical grade were 2.154 μg / bee and 18.944 μg / bee, respectively. It showed that the toxicity of formulation was markedly greater than that of technical grade by a factor of 4.6 and 8.8. The LD_5 of formulation were calculated as 1.085 μg / bee (Oral method) and 1.790 μg / bee (contact method). These values were used to treat the bees in the subsequent experiments.

3.2 Respiration rate and flight activity

The distribution of respiration rate wasn't the same across categories of exposure times ($n = 162$, $T = 29.643$, $df = 2$, $P < 0.0001$) and myclobutanil treatments (n =

162, T = 22.405, d f = 2, P < 0.0001) on forager bees. The respiration rate of workers exposed to myclobutanil was reduced when the exposure time was prolonged (Fig.1). Myclobutanil significantly reduced the respiration rate of workers at 24 h and 48 h after exposure compared with the control (F = 14.559, d f = 2, 69, P < 0.0001; F = 3.952, d f = 2, 69, P < 0.024), and there were no differences between contact exposure and oral ingestion treatments, but after exposure 3 h only oral ingestion of myclobutanil significantly reduced the respiration rate of workers (F = 8.164, d f = 2, 69, P = 0.001).

The number of worker from the treatments reaching the light source (120 cm) were less than the workers of control, neither contact exposure nor oral ingestion myclobutanil significantly affected the take-off flight (Fig.2).

3.3 Biochemistry assays

The effects of myclobutanil treatment on the ECOD and GST activities in the midguts of *A. cerana cerana* were shown in Fig.3. A significant increase for ECOD activity in nurse bees after exposure to the dose (LD_5) of formulation-grade myclobutanil was observed when compared to the corresponding control (F = 5.310, d f = 4, 10, P = 0.010) (A). There were no significant differences for ECOD activity in forager bees between treated groups and control (F = 2.583, d f = 4, 10, P = 0.102) (B). GST activities remained unchanged after being treated with the dose (LD_5) of formulation-grade myclobutanil both in nurse bees and forager bees. (nurse bees: F = 0.789, d f = 4, 15, P = 0.550; forager bees: F = 0.239, d f = 4, 12, P = 0.911) (C, D). Moreover, the dose effect was not significant for either nurse bees or forager bees between the LD_5 and LD_{50} treatment groups.

4. Discussion

In recent years, the application of agricultural fungicides has increased

dramatically [35], and the chance that honeybee exposed to the fungicide is increased as well. The triazole fungicides are widely sprayed onto blooming flowers of crops that are highly attractive to foraging bees. In general, such fungicides are considered to be safe to bees [36], but beekeepers have reported losses of brood in larval and pupal stages coinciding with fungicide use on almonds during bloom [37]. In the present study, formulation grade myclobutanil showed moderate toxicity to *A. cerana cerana* either contact (LD_{50} = 4.697 μg / bee) or oral (LD_{50} = 2.154 μg / bee) exposure. In addition, we found a significant difference in forager bee toxicity of the myclobutanil formulation and technical grade. The 48 h LD_{50} values of technical grade myclobutanil were 21.586 μg / bee for contact exposure, and 18.944 μg / bee for oral exposure, respectively. These results clearly demonstrated that the formulation ingredients [‘inerts’(co-formulants)] often increase the toxicity of the active ingredient for honey bees [11, 38, 39]. The reason may be the changes of physio-chemical properties of active ingredients in formulation by ‘inerts’, especially, the effective penetration of active ingredients into bees. However, the adverse effects of ‘inerts’ on bees are currently unknown. Furthermore, we found *A. cerana cerana* is more susceptible than that of *A. mellifera* (contact LD_{50}: 40 μg / bee; Oral LD_{50}: 34 μg / bee) to myclobutanil [15]. This result means that *A. cerana cerana* is a highly sensitive species to pesticide.

In addition to lethal effects, the present study revealed a variety of sublethal effects of myclobutanil on *A. cerana cerana*. Myclobutanil at sublethal dose significantly reduced the respiration rate of workers at 24 h and 48 h after exposure, clearly demonstrating that this fungicide can affect insect respiratory system. Respiration rate is an indicator of physiological stress, and insecticides can compromise insect respiration by impairing muscle activity, leading to paralysis [21,

40]. Here, we observed that myclobutanil didn't significantly affect the take-off flight. The reason for this result may be at the low level of myclobutanil, that occur in bodies of bees consuming this level of 12.5 % myclobutanil EC, minor inhibitory effects on flight muscle, may cause compensatory increases in antitoxic proteins, but this needs more study. To date, there are few reports on fungicide impairing pollinator activity, and little literature is available for comparison.

Cytochrome P450 is a kind of widely distributed protein superfamily, play a major role in phase I of biotransformation mechanisms being involved in the oxidation and detoxification of endogenous and xenobiotics compounds in insects and other animals [41]. The induction of cytochrome P450-dependent O-deethylation is extensively used as an indicator of exposure and response to organic pollutants in vertebrates [42]. In this study, ECOD activity in nurse bees was increased after exposed to formulation-grade myclobutanil (1.790 μg / bee). It is well known that EBI fungicides inhibit microsomal monooxygenases (P450), which is responsible for oxidative detoxication [10, 43]. Unexpectedly, an increased of ECOD activity after myclobutanil exposure was observed in our experiment. Similar result was obtained by Sergent et al [44], who reported that EBI fungicides can induce P450 enzyme activity increased in mammals. Johnson et al [6] also reported that EBI fungicides may induce P450 gene expression at low levels of exposure. It seems plausible that some fraction of the fungicide couldn't penetrate integument, only very small dose absorbed by midguts of *A. cerana cerana*. Moreover, transitory induction may also be related to endogenous mechanisms of homeostasis maintenance. In this work, ECOD activity responding to the low dose made from formulation-grade, but not responding to higher doses.

GSTs represent an important family of enzymes that play a central role in phase

Ⅱ of biotransformation mechanisms being involved in the detoxification of both endogenous and xenobiotics compounds, therefore, an induction of GST activity has been used as an environmental biomarker [25]. In this study, myclobutanil had no apparent effect on the GST activity. The response of GST, such as induction, inhibition and inactivation, may depend on the chemical stressor.

In forager bees, no enzyme markers response was obtained in this test. This may attribute to the fact that the dose absorbance is too less to induce some biochemical changes in the midgut of forager bees by using this topical exposure method.

Based on these results, it can be concluded that low dose of myclobutanil interfered with respiration and P450-mediated detoxification of the individual bees of *A. cerana cerana*. Thus it is likely to lead to interfere with depletion of energy available for flight and other activities. Future studies need to focus on the fungicide impacts at the colony level. Moreover, combination of EBI fungicides with insecticides has been revealed more deadly to the bees than either chemical alone. The molecular and physiological mechanisms of such interactions need to further research.

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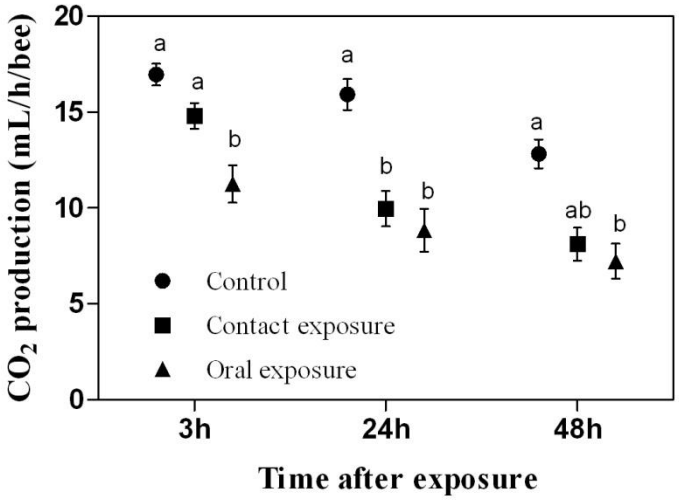

Fig. 1. Respiration rate (± SE) of forager bees of *Apis cerana cerana* 3 h, 24 h and 48 h after being exposure to myclobutanil. The different letters indicate significant difference myclobutanil treatments based on Tukey's HSD test ($p < 0.05$).

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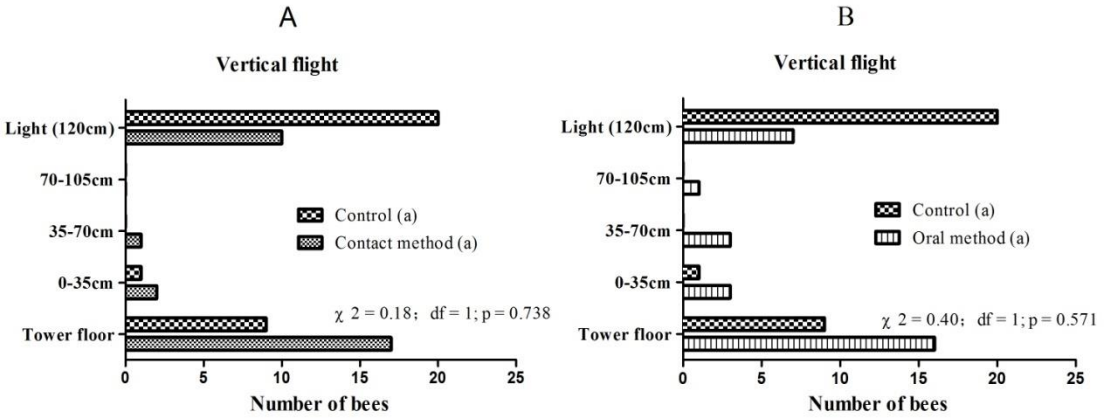

Fig.2. Flight activity of forager bees of *Apis cerana cerana* 24 h after exposed to myclobutanil (A contact; B Oral). The results of the (non-parametric) Kruskal-Wallis test ($P < 0.05$) used to test the differences between control and treatment. The legends in each chart followed by same letters in parentheses are not significantly different.

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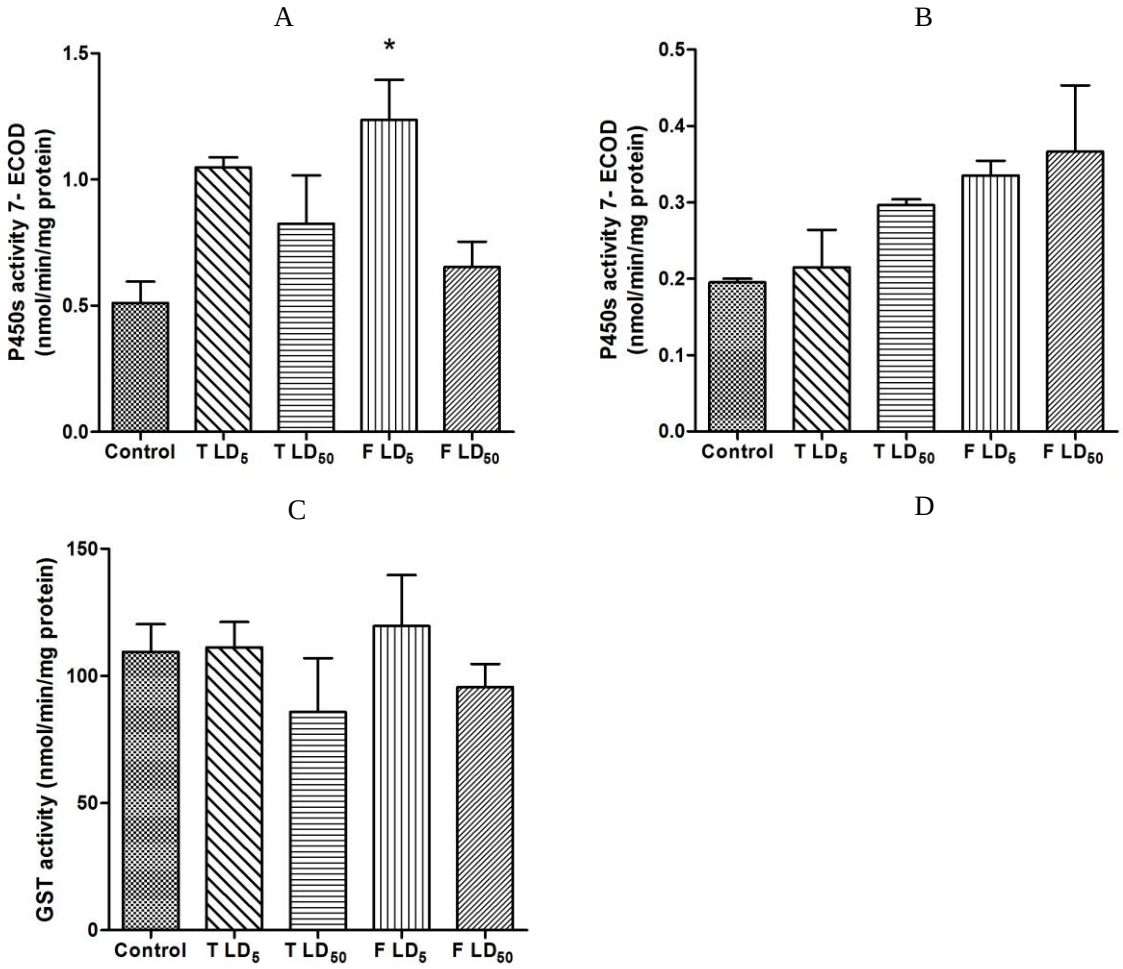
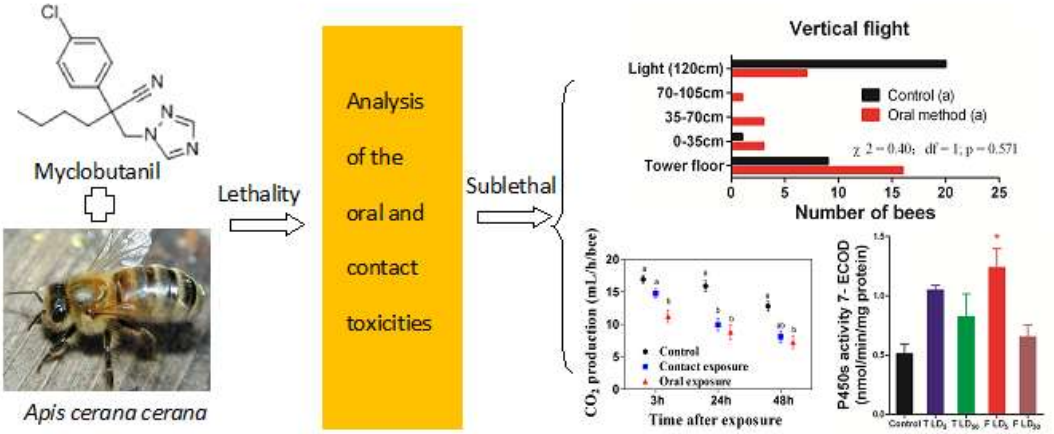


Fig.3. Effects of myclobutanil on the ECOD and GST activities in the midguts of nurse bee (A, C) and forager bee (B, D). Data represent the mean ± SE. "*" means significantly different from the control treatment (Tukey's HSD test $P < 0.05$). T LD_5 and T LD_{50} made from technical-grade myclobutanil. F LD_5 and F LD_{50}: made from formulation-grade myclobutanil.

Table 1 Oral and contact toxicities of myclobutanil to *Apis cerana cerana*

Pesticides	toxicities	Slope ± SE	LD_{50} (μg / bee) (95% CL) [a]	$(\chi^2)^b$	Sig	LD_5 (μg /bee)
Formulation (12.5% EC)	Oral	5.524 ± 0.973	2.154 (1.953 - 2.368)	0.528	0.913	1.085
	Contact	3.925 ± 0.565	4.697 (4.003 - 5.393)	1.182	0.757	1.790
Technical (purity 96.2%)	Oral	1.179 ± 0341	18.944 (10.763 – 29.301)	0.025	0.999	—
	Contact	3.355± 0.511	21.586 (18.409 - 25.526)	0.094	0.993	—

[a] Lethal dose causing 50 % mortality after 48 h with 95 % confidence limits; [b] Chi square



Graphical abstract

Highlights

- Myclobutanil is moderate toxic to adult Chinese honeybee *Apis cerana cerana*.
- Myclobutanil through contact exposure and oral ingestion impaired worker respiration.
- Myclobutanil didn't significantly affect the take-off flight.
- Myclobutanil affected P450 activity of nurse bee worker.
- Myclobutanil may has risk for *Apis cerana cerana*, it should be cautiously used.