Effect of sub-lethal dosages of insecticides on honeybee behavior and physiology

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Abstract

Honeybee, *Apis mellifera*, is one of the most important pollinators in the world. Since pesticides have been used widely, honeybees are unavoidable at risk exposing to various contaminated sources of residual pesticides while they are engaging foraging activities in collecting nectar and pollens. The effects of various levels of sub-lethal dosages of insecticides on the biology, development and foraging activities of honeybee would not be direct reflected by the LD_{50} of the acute toxicity of insecticides. In this paper, we review available information and our preliminary studies of some commonly used insecticides, especially pyriproxyfen and imidacloprid affect the development, foraging behavior and colony conditions of honeybee under sub-lethal dosages.

Keywords: honeybee, insecticide, sub-lethal dosage, pyriproxyfen, imidacloprid

1. Insecticides and the intoxication of bees

Insecticides are important for ensuring both crop quality and quantity in today's integrated crop management for sustainable agricultural production. The use of insecticides is one of the most effective practices to control pests. However, what concerning us most is how residual levels of sub-lethal dosages of those insecticides being used resulted in detrimental effect on non-target pollination species of honeybee its development, foraging behavior and colony conditions. Either wild or domesticated honeybee, *Apis mellifera*, has been recognized and used as a major pollinator in the agricultural system (Kevan 1999) and by beekeepers to produce valuable products such as honey, royal jelly and pollen. However, honeybee rely on flower plants while foraging and collecting its food sources of nectar and pollen and thus at risk endangering exposing to various levels of chemical residues of pesticides while they are collect nectar and pollen (Peach et al. 1993). Honeybee workers may be poisoned by the residual pesticides on the nectar and pollen they collect. In addition, the workers may take the pesticide-contaminated nectar and pollen back to their hive. This will expose the larvae, drones and queen to these pesticides, and eventually poison them and causes high mortality.

The toxicity of insecticides use animal experiments to estimate the half lethal dosage (LD_{50}) or lethal concentration (LC_{50}), and thus estimate the possible harm to humans and non-target organisms. To non-target organisms, insecticides not only can cause the direct poisoning/death of bees directly, it can also influence the bee larvae, division of labor, foraging, as well as the development of bee colonies while subjecting them to a lower lethal dose (EPPO, 1993, Thompson

2003). So it is important to investigate the effects of insecticides on bees below the estimated lethal dosages or concentrations, which is called sub-lethal dosages or concentrations (Haynes 1988, Desneux et al. 2006). Previous studies have shown that low-dosage deltamethrin will delay the return time (Vandame et al. 1995) and reduce the foraging activity (Decourtye et al. 2004). Also, cypermethrin leads to disappeared bees (Bendahou et al. 1999), while parathion influences the communication between bees (Schricker and Stephen 1970). In addition, fipronil, Cypermethrin, prochloraz, and endosulfan influence olfactory learning performance (Decourtye et al. 2005). All of the above mentioned insecticides may cause abnormal behavior, broken colonies, and reduce bee products, resulting in economic losses (Thompson 2003). Therefore, the effect of insecticides on the direction sense of bees (homing ability), their communicative ability (bee dance language), and their foraging activity (olfactory learning performance) are more important than the LD_{50} of insecticides (Pham-Delègue et al. 2002).

2. The effect of juvenile hormone analogue on bees

Over the past decades, insect growth regulators (IGRs) have been developed and are being widely used for pest control, simply because of their high potency and selectivity for insects and with a low toxicity to mammals. However, when it comes to the safety of the honeybee, the residue of IGRs remaining in the field is a chronic killer for the honeybees. Pyriproxyfen (Sumilarv) is one of these IGRs and has been widely used against some arthropods since the early 1990s. Pyriproxyfen is classified as a juvenile hormone (JH) analog, and its molecule possesses only little similarity to endocrines JH, still it affects JH and ecdysteroid titers in arthropods (Bitondi et al. 1998, Zufelato et al. 2000).

JHs are a mixture of three terpenoids. They are distributed into four types: C-19 juvenile hormone (JH-0), C-18 juvenile hormone (JH-I), C-17 juvenile hormone (JH-II), and C-16 juvenile hormone (JH-III), with JH-III being the most common type (Bergot et al. 1980, Richard et al. 1989). However, researchers only found JH-III in *A. mellifera* (Hagenguth and Rembold 1978). The allatotropin and allatostatin is secreted by neurosecretory cells in the brain of the insects in order to control the corpora allata secreting JH (Wigglesworth 1952, Tobe and Stay 1985). The major functions are to regulate the metamorphosis, the behavior and development (Riddiford 1994), and it correlates with cuticular melanization and maturity at the pupal phase (Hiruma et al. 1993).

Workers carry out tasks in accordance with the age polyethism in the hive. The younger workers (1 to 3-week-old) work inside the hive such as cleaning, food storing, and larvae and queen caring, while the older ones (> 3-week-old) work outside the hive, carrying out tasks such as forage and defense (Winston 1987). JHs play an important role in age polyethism (Jaycox et al. 1974, Robinson 1985, Huang et al. 1994, Robinson and Vargo 1997). The quantity of JHs in a hemolymph increases with the age of the bee. In addition, the younger bees, which still work inside the hive contain a smaller amount of JH, while the older bee that works in the field contains more JH (Fluri et al. 1982). The JH, juvenile hormone mimic, or the juvenile hormone analogue is topically applied on the dorsal thorax of the new eclosion bee, and as a result it turns earlier into a forager (Jaycox et al. 1974, Huang et al. 1991, Robinson and Vargo 1997). The foraging behavior between the early bee and the normal bee shows no difference (Deng and Waddington 1997). At the same time, the house bee will turn into a forager late if the corpora allata is eliminated (Sullivan et al. 1996). The age polyethism of workers is changed by the demand of the bee colony (Page et al.

1992, Huang and Robinson 1995), and can accelerate or decelerate the behavioral development, even the transition from forager to nurse bee (Robinson 1992). JH can adjust the development to adulthood, by effecting the shifts from larvae/larvae and larvae/pupae (Zhou and Riddiford 2002). In addition, JH can influence the caste differentiation in the bees (Rachinsky et al. 1990).

Pyriproxyfen is like JH which affects the hormonal balance in insects and inhibits embryogenesis, egg hatch, metamorphosis, and adult eclosion, and causes the death of the insects (Glancey et al. 1990, Reimer et al. 1991, Miyamoto et al. 1993). Although pyriproxyfen is an analog, it has a better ability to compete for the JH receptor site because the potency of pyriproxyfen is more powerful than natural JH (Cusson et al. 1994). Because pyriproxyfen has high level of potency and selectivity for insects and a low level of toxicity for mammals, it is suitable for controlling pests such as houseflies, mosquitoes, and cockroaches, and is used in husbandry and gardening, where it prevents mainly pests such as mealworms, aphids, and coccids.

It has been found that pyriproxyfen has low toxicity for adult worker bees. The LD_{50} of pyriproxyfen for a honeybee is more than 100 μg/bee (WHO 2001), and is therefore considered as non-toxic to bees. De Wael et al. (1995) found that bumblebee, *Bombus terrestris*, colonies developed normally after feeding on pyriproxyfen syrup. However, as mentioned earlier, the honeybee workers may take the pesticide-contaminated food back to their hive and feed the larvae. It is the effect of pyriproxyfen on the development of the larval phase that is crucial.. When the larvae of the honeybee workers are treated with pyriproxyfen at different periods, both the larval and pupal developments are retarded and the adult emergence rate declines (Bitondi et al. 1998, Zufelato et al. 2000). Since pyriproxyfen is harmful to the development of honeybee larvae and pupae, one can assume that the honeybee colony will collapse gradually when contaminated by pyriproxyfen.

In our study, 1-day-old larvae were reared in laboratory and 0.1 - 100 ppm pyriproxyfen was added in larval food in the artificial rearing larval honeybee method. A dose respondent effect was found in the larval development of the treated larvae (Table 1). Results showed that 0.1 ppm pyriproxyfen reduced the eclosion rate of the reared larvae conspicuously, and 1 ppm pyriproxyfen reduced that 84.6% of eclosion honeybee appeared the condition of deformed wings. The 10 ppm pyriproxyfen treatment caused 53.8% melanic death in the pupal stage, and no honeybees succeed in eclosion. Adding 100 ppm pyriproxyfen in larval food caused 50% death in larval stage, and no honeybees succeed in eclosion, too.

3. The effect of neurotoxic insecticides on bees

Ninety percent of insecticides are neurotoxins, and preventing neural transmission is the main mechanism for killing insects (Raymond-Delpech et al. 2005). The neurotoxic insecticides are divided into five groups: organophosphorus compounds, methylcarbamates, organochlorines, synthetic pyrethroids, and neonicotinoids. Neonicotinoids have low toxicity for mammals and are considered a perfect insecticide. Neonicotinoid insecticides act as an agonist of acetylcholine (ACh) to occupy the binding site of ACh nicotinic receptors in the central nervous system causing excitation and eventually paralysis leading to death (Bai et al. 1991, Buckingham et al. 1997, Tomizawa and Casida 2005).

Imidacloprid 〔 1- (6-chloro-3-pyridylmethyl)-2- nitroimino-imidazolidine 〕 is a neonicotinoid insecticide with a high potency and selectivity against insects but low toxicity for

mammal (Elbert et al. 1991). In addition, it has a high stability in light and is one of the important insecticides in the control and prevention of pests (Liu and Casida 1993, Tomizawa and Casida 2003).

Imidacloprid is a systemic insecticide of oral and contact toxicity (Elber et al. 1990) that can protect plants from pests by spraying leaves, seeds and the soil (Elbert et al. 1991). The seeds treated with the systemic insecticide imidacloprid are called Gaucho® seed (Bayer). This seed-dressing was first used on sunflowers in France in 1994. However, in 1996 a novel bee malady was reported which became aggravated the following years (Schmuck 1999, Schmuck et al. 2001), and the effect of imidacloprid was suspected to be responsible.

Previous studies have found that the acute oral LD_{50} values of imidacloprid on honey bee were 3.7~81 ng/bee (Elbert et al. 1991, Schmuck 1999, Suchail et al. 2000, 2001, Schmuck et al. 2001), and the acute contact LD_{50} values were 59.7~242.6 ng/bee (Suchail et al. 2000, Schmuck et al. 2001). For chronic toxicity, the chronic LD_{50} of imidacloprid on honey bee was 0.01 ng/bee (Suchail et al, 2001).

The present investigation of the effect of imidacloprid on bee behavior found the following:

3.1 The effect of imidacloprid on the foraging behavior of honeybees

The foraging behavior of honeybee affects the rate of foraging, the probability of returning, and the to and fro times due to the concentration of sucrose (Scheiner et al. 2004). Previous studies shows that the rate of returning forager bees is reduced when honeybees are fed on 50% syrup with 6 μg/kg (Colin et al. 2004), 20 ppb (Kirchner 1999), 24 μg/kg (Decourtye et al. 2004), 48 μg/kg (Ramirez-Romero et al. 2005), 500 ppb, or 1000 ppb imidacloprid (Bortolotti et al. 2003).

3.2 The effect of imidacloprid on the learning ability of honeybees

The learning abilities of the honeybee can be assessed using the conditioning of the proboscis extension reflex (PER). Decourtye et al. (2003) used the principle of classical conditioning (Takeda 1961) which combines PER with flavor and olfactory learning ability, and the result showed that there was a decrease in olfactory learning ability when honeybees were treated with 12 μg/kg, 24 μg/kg, 48 μg/kg, or 96 μg/kg imidacloprid.

3.3 The effect of imidacloprid on the activity of honeybees

Although take a sub-lethal dose of imidacloprid will not lead to the immediate death of a honeybee, it affects the foraging behavior (Kirchner 1999, Bortolotti et al. 2003, Colin et al. 2004, Decourtye et al. 2004, Ramirez-Romero et al. 2005) and reduces the learning ability (Decourtye et al. 2003). Medrzycki et al. (2003) found that only 20 ppb of imidacloprid was enough to decrease the foraging activity in honey bee colonies and that the foraging behavior was suppressed at levels above 100 ppb after 30 - 60 min.

3.4 The effects of imidacloprid on the homing rate of honeybees

Bortolotti et al. (2003) demonstrated that honey bee workers were confused and disoriented if they were treated with imidacloprid. A sub-lethal dose of imidacloprid in sucrose solution affects both the homing ability and the foraging activity of honey bees, and only 500 to 1000 ppb of insecticide in syrup was sufficient to cause the workers to fail to return to their hive or to the feeding site. In addition, an imidacloprid solution as low as 100 ppb could delay honey bee workers for up to 24 h from returning to their hive or arriving at their feeding site.

3.5 The effect of imidacloprid on bee colonies

If the forager bees contact plants containing imidacloprid outside their hive, they will take the contaminated nectar and pollen home with them, and the nurse bees will feed the larvae contaminated food. Schmuck et al. (2001) fed bee colonies on sunflower nectar that contained 2, 5, 10, and 20 μg/kg imidacloprid for 39 days. The results showed that it did not affect the mortality rate of the honeybees, nor their foraging behavior, nor the quantity of beeswax that the hive produced, and the ability of the nurse bees and the worker bees under the highest concentration (20 μg/kg) treatment were not affected. These results indicate that imidacloprid at low concentration will not affect bee colonies when the concentration is below 10 ppb in the soil, nectar, and pollen of the natural environment (Schmuck 1999, Schmuck et al. 2001, Wallner 2001, Laurent and Rathahao 2003, Bonmatin et al. 2005). However, it is probable that the content of imidacloprid accumulates in the hive and affects colonies when workers forage the contaminated food repeatedly and over a longer period of time.

In our recent study (Yang et al. 2008), we observed the time interval between two visits of the same honeybee at the same feeding site. Under the normal situation, the time interval was less than 300 seconds. However, 15.2% of the bees showed abnormal foraging behavior (>300 sec.) when the concentration of imidacloprid was higher than 50 μg/L. At concentration of 600 μg/L, 34.4% of the tested bees were missing, and the number of missing bees would increase with the concentration. Abnormal foraging behavior was observed even if these missing bees were back to the feeding site on the second day.

In addition to the honeybee adults, the survival, capped-brood, pupation and eclosion rates were also observed after treating the larvae directly in the hive with imidacloprid in different dosages. The capped-brood, pupation and eclosion rates significantly decreased when the dosages increased from 2000 to 8000 ng (Fig. 1).

Conclusion

The development of intensive agriculture in recent decades has resulted in the extensive use of insecticides. This has led to the sharp decline of pollinators. Honeybees are one of the most important pollinators, and a decline in their numbers will cause serious pollination problems if plants have to do without the high efficiency of pollination by honeybees. It will be especially devastating for the propagation of wild plants, and it may cause whole system changes and declines in the flora on the earth. It is evident that honeybees are not only indispensable to agriculture, but they are critically important to the natural balance of our ecosystem.

There is a tendency in the development of insecticides to reduce risks, to give a high priority to developing insecticides with high security, and those that only require the use of very small quantities and those that are highly specific. Pyriproxyfen and imidacloprid conform to these three pre-requisites, they have high potency and high selectivity against insects and they have a low toxicity to mammals. Consequently they are deemed to be perfect insecticides. Yet, both pyriproxyfen and imidacloprid are still harmful to the honeybees.

Bees are eusocial insects, and they are interdependent at different stages. Thus, whichever stage encounters toxicity; it could induce the bee colony to collapse. It is worth noting that the

earlier studies all treated the adult bee, and they were not concerned with the larvae bee. But, a bee colony is maintained by bees of different ages and by bees in different stages (Seeley 1982, Calderone and Page 1992). In other words, it is not only the foragers that can readily have contact with residual insecticides on plants. Other members of the hive are affected as well, since the contaminated nectar and pollen is carried to the hive by the forager, thereby affecting the entire bee colony. Many pesticides have low toxicity for adult insects,. For example, the insect growth regulator has almost no toxicity to adult insects, but by blocking larval development to it reduce the insect pests. It is for precisely this reason that we can not sufficiently estimate the effect of pesticides on honeybee using our present toxicity tests. Most pesticides presently in use have no effect on the mortality of adult honeybee, but their effect on larvae is not. It is crucial that we continue to investigate the effect on bee colony development using sub-lethal dosages of pesticides.

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Table1. The ratio of abnormal development of basic larval food with pyriproxyfen on artificial rearing larval honeybees

Pyriproxyfen in BLD	Larvae succeed to pupation (%)*	Pupal death		Adult emergence		
		Black (%)	Other (%)	Total (%)	Normal (%)	Deformed wings (%)
100 ppm	0.38 b**	0 c	0.38 b	0	0	0
10 ppm	55.1 a	53.8 a	1.3 b	0	0	0
1 ppm	68.7 a	49.7 a	3.5 a	15.5 b	2.4 b	13.1 a
0.1 ppm	58.9 a	1.5 b	4.8 a	52.6 a	49.8 a	2.8 b
0 ppm	67.2 a	0 c	5.3 a	61.9 a	60.5 a	1.4 b

*Each assay contained 33-48 larvae in a colony and was performed with 8 colonies.

** Means ± s.d. in each same column followed by different letters are significantly different by the LSD test ($P < 0.05$).

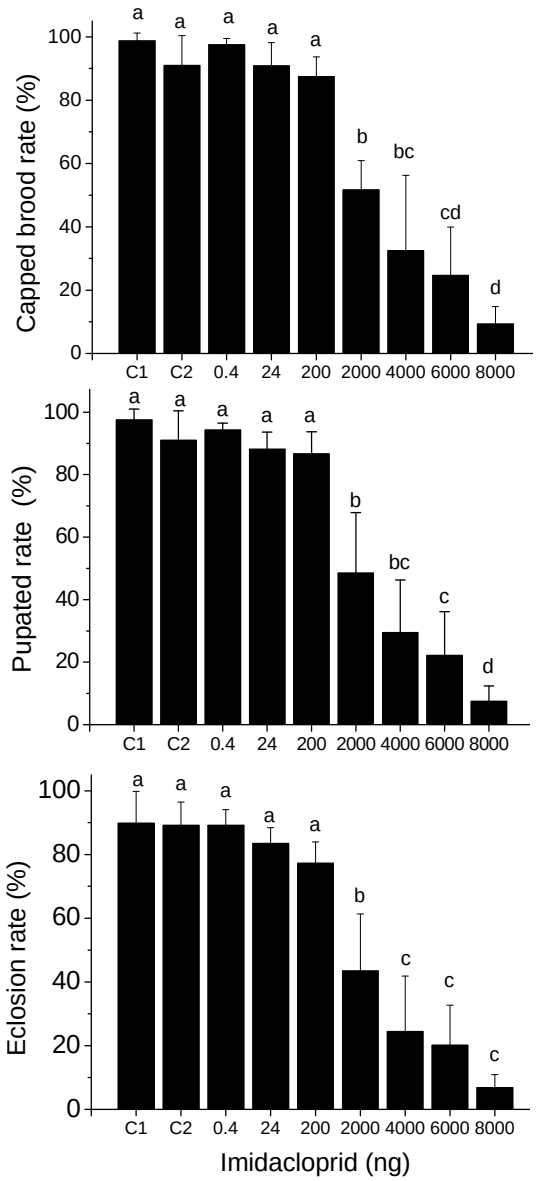

Figure 1. Effect of the imidacloprid on (A) the capped-brood rate (B) the pupate rate, and (C) the eclosion rate of honeybee larva.The dosage of imidacloprid were 0.4, 24, 200, 2000, 4000, 6000 and 8000 ng. C1 and C2 were control group (0.1 and 1% DMSO). The different letters indicate no significantly difference. (N = 4 colonies, n = 30)