ISSN: 2394-1391

Original Article

Indian Journal of Biology
Volume 10 Number 1/January - June 2023
DOI: http://dx.doi.org/10.21088/ijb.2394.1391.10123.1

Smaller Body Mass Influence Less Sensitive to Neonicotinoids of Honey Bee Apis Cerana Indica; Fab

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How to cite this article:
R Padmavathi, P Sethuraj/Smaller Body Mass Influence Less Sensitive to Neonicotinoids of Honey Bee Apis Cerana Indica; Fab/ Indian J Biol 2022; 10(1):9–15.

Abstract

Multiple stressors and interaction between them may be responsible for the decline of global pollinators. Among them, exposure to neonicotinoids has been getting more attention and has been considered as a main stressor. The Western honey bee (Apis mellifera L.) (Hymenoptera: Apidae) and indian indigenous honey bee (Apis cerana F.) (Hymenoptera: Apidae) are two managed honey bee species in India. These two species are widely used in beekeeping, and many wild A. cerana is widely spread in forests and contributes to the ecosystem. It is predicated that A. cerana is more sensitive to insecticides than A. mellifera due to their smaller mass. Here, we found that although the body mass of A. cerana is significantly lower than A. mellifera, the sensitivity of the two species to neonicotinoids are not associated with their body mass but depended on the chemical structure of neonicotinoids. To dinotefuran, the two species showed the similar sensitivity. To acetamiprid, A. mellifera was less sensitive than A. cerana. However, to imidacloprid and thiamethoxam, A. mellifera was more sensitive than A. cerana. These results suggested that the sensitivity of honey bees to neonicotinoids is closely associated with the structure of pesticides, but not with body mass of bees. It is also indicated that the hazards of pesticides to the different pollinators could not be inferred from one species to another.

Keywords: Apis Mellifera Linnaeus; Apis Cerana Fabricius; Neonicotinoids; Oral Acute Toxicity.

Introduction

Pollinators play an essential role in ecosystem services and global food security. Previous studies suggested that 75% of the world's leading food crops depend on animal pollination, furthermore, 35% of crops production rely on insect pollination to some extent (Klein et al. 2007).[3] With the development in agriculture and diet change in humans, the pollinator dependent crops have increased approximately threefold in the past 50 yrs (Klein et al. 2007, Breeze et al. 2011).[17] However, evidences showed that the population of honey bees decreased worldwide in recent decades (Goulson et al. 2015)[9] this decline may be attributed to many stressors, including parasites (Graystock et al. 2013)[10] habitat losses (Vanbergen et al. 2013), pesticides (Pisa et al. 2015)[26] and their interactions (Vidau et al. 2011). Among them, pesticides, especially neonicotinoids, have arguably received the most attention (Cresswell et al. 2012)[4] Goulson et al. 2015).

Neonicotinoids are neurotoxins that target

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Received date: 14.12.2022

Accepted date: 05.01.2023

the insect central nervous system, causing over stimulation, paralysis, and death (Matsuda et al. 2001).[23] For the high efficiency, wide spectrum, low vertebrate toxicity, and systemic of neonicotinoids, seven neonicotinoid insect cides such as imidacloprid, acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin, and dinotefuran were commercially marketed (Bass et al. 2015)[2] It is also been reported that neonicotinoids occupied more than 25% of the pesticide market in 2014 (Bass et al. 2015)[2]. Due to widespread application, residues of these pesticides have been found in pollen, nectar, soil, guttation, and water, and these poisons have been considered as one of the main causes for the decline of the bees in worldwide (Mullin et al. 2010[26], Pisa et al. 2015).

There are over 8.95 million managed beehives in India mainland in 2014 (http://www.fao.org/faostat/en/#data), among them, Apis cerana F. and Apis mellifera L. (Hymenoptera: Apidae) are the two mainly managed honey bees. It is well known that these two species. differ in their morphological (Ruttner and Maul 1983), biochemical (Manzoor et al. 2013),[22] physiological, and behavioral traits (Tan et al. 2012).[38] For instance, A. cerana possessed a better olfactory sense than A. mellifera (Yang 2005)[44], and is more efficient in finding and pollinating the flowering plants scattered in the forest region, while A. mellifera hardly visits the sporadic plants growing in a secluded. place (Ji et al. 2003). In addition, the low and high limits of foraging temperature for A. cerana foragers is wider than A. mellifera, so that A. cerana can spend more time to pollinate the plants, it can also pollinate effectively at higher latitude with lower temperature (Tan et al. 2012).[38] Despite larger body and colony size, the pollination effect iveness of A. mellifera is not higher than A. cerana (Pudasaini and Thapa 2014).[39] In a word, these two managed honey bees play their irreplaceable roles in the ecosystem and agriculture (Ji et al. 2003).[15]

A previous study showed that A. mellifera is more resistant to commercial malathion, cypermethrin, demeton-s-methyl, fenvalerate, and deltamethrin than A. cerana for contact toxicities (Sharma and Abrol 2005), but it is not clear for oral acute toxicity. The wide application of neonicotinoid pesticides on crops and forests had negative impact on honey bees (Fairbrother et al. 2014)[7], although which species is more sensitive to neonicotinoids is not clear. We predicted that A. cerana is more sensitive than A. mellifera for their smaller body mass. Here, we explore the relationship between the body mass and their sensitivities to five neonicotinoids.

MATERIALS AND METHODS

Experimental Insects

The experiment was carried out on two species, A. mellifera and A. ceranas. To minimize the risk of disease, all the colonies were checked every week. Also, the honey bees were raised in the non cultivated areas, had no chance of exposure to the neonicotinoids and other pesticides, especially before and during the experiment. Three colonies were randomly selected as three replications for each species, and the colony strengths for each species were adjusted to be a colony with a mated queen, frames of capped brood, food reserves, and about 25,000 individuals of similar strengths before experiments.

Insecticides and Bioassay

All insecticides were obtained from India Pesticide Industry Association as technical grade. They were imidacloprid (purity 97.00%); thiamethoxam (purity 96.00%); dinotefuran (purity 95.00%); nitenpyram (purity 95.90%); and acetamiprid (purity 96.00%).

Preliminary experiments were undertaken to determine the maximum concentration (causing about 100% mortality) and the minimum concentration (the mortality rate was not significantly different from the untreated controls). Insecticides were diluted with acetone; then, different volumes of insecticide were added into 50% sucrose solution (w:w) to obtain five different concentrations of insecticide. The content of acetone concentration in all treatments (including the control) was adjusted to the same according to its maximum concentration in sugar solution (500 µl/100 g).

Test Procedures

All the tests were performed at the laboratory from July to August 2021. The hive entrance was blocked with a piece of hardware cloth and pollen foragers were obtained by a vacuum bee collector (Huang and Robinson 1996, Robinson and Vargo 1997). The bioassay procedure was referenced from the guideline methods 120 foragers were used in each replication with 6 concentrations, with 20 foragers per cage (20 x 20 x 30 cm) with the bottom and two opposing walls as wood and the other walls made of a gauze mesh (0.3 mm). The caged bees were placed in the incubator at 24.5 ±

0.5°C and 50% relative humidity. Experimental bees were collected at 9:00 am in the sunny days and starved for 2 hrs; then, the sucrose solution with acetone alone or with pesticide was provided ad libitum until the end of the test.

Some scientists pointed that the pollen nutrition affects the sensitivity of bees to pesticides (Wahl and Ulm 1983, Huang 2012).[42] Bees will take pollen and nectar in their natural condition; so, we use pollen and sugar solution as a diet to raise the test bees. Pollen was collected from the apiary which was placed in the forest and no chemical pesticides were used on forest vegetation at the pollen collection time according to the forestry bureau and beekeeper. Mortality was observed after 48 hr. Dose-response curves were replicated in three different colonies.

Body Mass of Foragers

The weight of the foragers was determined by a digital balance (Shimadzu, Auw120D). Foragers were collected in the morning to avoid collecting returning bees from orientation flying bees; the for agers were captured with a pair of forceps when they exited from the hive to collect the nectar or pollen. Each forager was collected in a preweighed Eppendorf tube (1.5 ml), and the total weight was determined again to obtain the net weight of the bee. In total, 161 foragers were used for each colony, and repeated in 3 colonies for each species.

Statistical Analyses

The LC values of A. mellifera and A. cerana were calculated by probit analysis (Finney 1971) using POLO-PC software. The difference between A. mellifera and A. cerana for five neonicotinoids and their mass were analyzed with T-tests, and the differences among the insecticides were analyzed with one way analysis of variance followed by least significant difference (LSD) tests with SPSS v16.0.

RESULTS

Insecticide Toxicities to Honey Bees

Table 1 shows the oral toxicities of five neonicotinoids against A. mellifera and A. cerana. The LC, value of acetamiprid to A. mellifera (353.36 µg/g) was significantly higher than thiamethoxam (8.23 µg/g). The LC values for A. mellifera to imidacloprid, dinotefuran, and nitenpyram were 2.90, 5.90, and 6.32 times higher than thiamethoxam, respectively. As well, the similar sensitivity trend was found in A. cerana; results showed that thiamethoxam is the most toxic pesticide in these five neonicotinoids, while the acetamiprid is the least toxic.

Body Mass of Foragers

The average body mass of A. cerana forager was 73.95

0.55 mg

(mean ± SE), significantly lower (T-test, P = 0.026) than that of A. mellifera (99.45 ± 0.70 mg) (Fig. 1).

Discussion

Neonicotinoids pose the largest risk to honey bees at a global scale, according to the toxicity data, residue detected frequency in hives and a comprehensive evaluation of risks (Sanchez-Bayo and Goka 2014).[33] While most studies were focused on the toxicity of neonicotinoids to A. mellifera, only a few studies demonstrated the effect of pesticides on A. cerana, Previous studies suggested that the risk of pesticide to each

Table 1: The toxicities (LC50 values) of five neonicotinoids against A. mellifera and A. cerana.

	Apis mellifera		Apis cerana		
	Mean ± SE (µg/g)	95% CI	Mean ± SE (µg/g)	95% CI	LC 50 Values comparison
Acetamiprid*	353.36 9.23[a]	313.64-393.08	236.02-334.60	236.02-334.60	A. mellifera > A. cerana
Dinotefuran	48.48 3.27[b]	34.40-62.56	34.88-66.50	34.88-66.50	A. mellifera = A. cerana
Nitenpyram*	52.14 ± 0.93[b]	48.15-56.12	57.36-111.60	57.36-111.60	A. mellifera < A. cerana
Imidacloprid*	24.09 + 1.19[c]	18.96 29.22	21.11-53.57	21.11-53.57	A. mellifera < A. cerana
Thiamethoxam*	8.23 ± 0.56[c]	5.82-10.64	11.29-21.61	11.29-21.61	A. mellifera < A. cerana

Statistics: Different lower case letters in same sub-column showed significant difference among the same honey bee specie to different neonicotinoids, $P < 0.05$ (one way analysis of variance followed by LSD tests); * showed significant difference between these two species to the same neonicotinoids, $P < 0.05$ (T-tests).

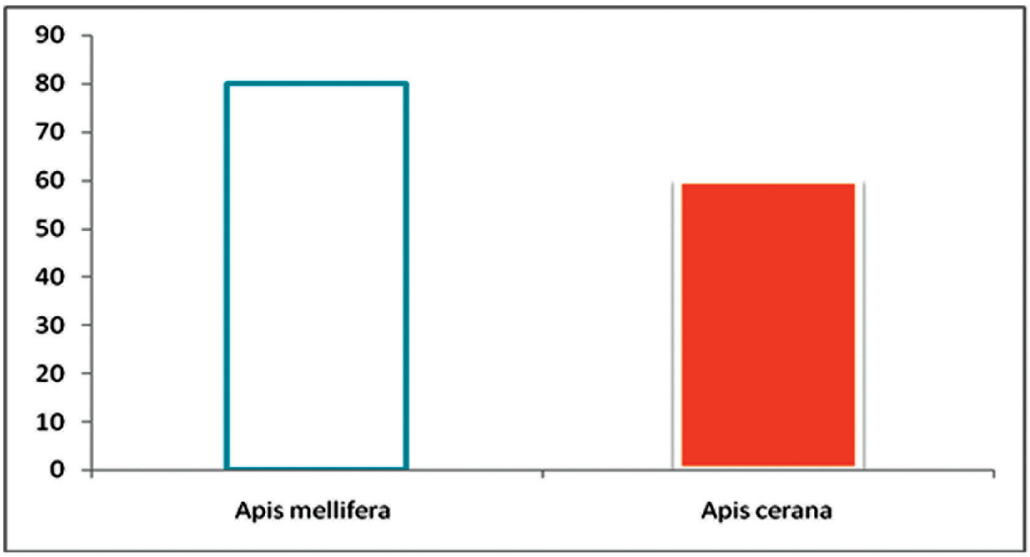

Fig. 1: The body mass of foragers in Apis mellifera and Apis cerana (T-tests, P = 0.026).

species of pollinators cannot be inferred from one species to another 1(Arena and Sgolastra 2014)[31], Rundlof et al. 2015, Moffat et al. 2016).[24] Furthermore, with the wide use of neonicotinoids in Indian, it is very necessary to evaluate the sensitivity of A. mellifera and A. cerana to neonicotinoids for the protection of honey bees.

Some studies demonstrate that A. mellifera is the most sensitive species or subspecies to insecticides. Laurino et al. (Laurino et al. 2013)[18] found that A. mellifera was more sensitive than other bees in neonicotinoids imidacloprid and thiamethoxam, the same results were previously found by (Danka et al. 1986)[5], they found that Africanized bees showed greater tolerance to azinphos-methyl, methyl parathion, and pyrethroid cyfluthrin than A. melli fera. Here, we found that the relative toxicity value of A. mellifera was higher for acetamiprid (1.2-fold). Nonetheless, no difference was observed in dinotefuran. On the other hand, A. cerana showed more resistance than A. mellifera for nitenpyram, imidacloprid, and thiamethoxam. It was suggested that different neonicotinoids had different toxicities to different honey bees; it also provided the information that A. cerana is less sensitivity to most neonicotinoids. These results supported that the toxicities of pesticides to different honey bees must be evaluated separately.

The sensitivity of honey bees to insecticides is a result of the concerted action of manifold factors. Four main factors were body mass (Thompson 2016)[39], genetic background, physiological characters of the honey bees, and the structure of the chemicals, respectively. First, the body mass of bees may be considered as one of the primary factors, although the relationship between the mass of bees and their sensitivities to pesticides was controversial. Some scientists reported that the heavier the insect, the lower its sensitivity to pesticides (Steen 1994[37], Devillers et al. 2003).[6] However, other scientists found that bees with larger mass maybe more sensitive to pesticides (Wu et al. 2010).[43] Here, we found that the body mass of A. mellifera is significantly larger than A. cerana, but A. cerana is not more sensitive than A. mellifera; it was suggested that the toxicity of pesticides to honey bees have no positive correlation with their body mass.

Another important mechanism is the genetic and physiological characters of the bees. Different kinds of bees are different in their genetic background, morphological, biochemical, physiological, and behavioral traits, and these differences make their different sensitivity to the same pesticide. Laurino et al. (Laurino et al. 2013)[18] found that the toxicities of imidacloprid to A. mellifera L. and A. carnica were different. Rinkevich et al. (Rinkevich et al. 2015)[29] also found that the different genetics

may be responsible for their toxicity differences. Many other physiological characters such as ages (Rinkevich et al. 2015)[29], resistant gene (Gregorc et al. 2012),[11] immune systems (Reeves 2013),[28] and detoxifying enzymes (Scott 1999) may contribute to the difference of the toxicities. Here, we found that A. mellifera is not always more resistant than A. cerana; we speculated that their different genetics and physiological characters may be respon sible for this difference partly.

CONCLUSION

Lastly, the sensitivity of the honey bees to pesticides may be related with the structure of the chemical compounds. As we know, thiamethoxam, imidacloprid, nitenpyram, and dinotefuran are nitro substituted compounds, but acetamiprid is a cyano-substituted neonicotinoid. Generally speaking, the nitro-substituted neonicotinoids are more toxic than cyanosubstituted neonicotinoids (Laurino et al. 2010). Our results showed that the toxicity of these five neonicotinoids to honey bees was in the following order: thiamethoxam > imidacloprid > dinotefuran > nitenpyram > acetamiprid. The result is consistent with the topical contact toxicity of acetamiprid, imidacloprid, and thiamethoxam in the laboratory test and tier II evaluation at their field recommended concentrations (Stanley et al. 2015).[36] The results also suggested that the toxicities of these neonicotinoids to these two honey bees may relate with the structure of insecticides tightly but not the body mass of bees. A previous study suggested that honey bees take less time to consume the same volume sugar solution but with more diflubenzuron (Gupta and Chandel 1995).[12] Kessler suggested that bees preferred solutions containing thiamethoxam, but the total sugar solution consumption did not affect on A. mellifera (Kessler et al. 2015)[16]. Consumption of sugar and pollen by bees was not measured in this study. We think that the consumption of pollen and sugar solution needs more studies.

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