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Evaluation of acute contact toxicity of imidacloprid to *Apis mellifera* under laboratory conditions

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Abstract

Neonicotinyl, systemic insecticides are very popular amongst the growers due to their low mammalian toxicity and the ability to move systemically into the entire plants, observed to be highly toxic to bees and are well known causal of colony collapse disorder in beekeeping industry throughout the world. In this communication, evaluation of imidacloprid toxicity to assess the risk posed to honey bees in field young worker bees of *A. mellifera* were carried out using hand operated micro-applicator under laboratory at 25 ± 2 °C and 65 ± 5 relative humidity in Sloan, Himachal Pradesh during august 2017. The toxicological investigations carried out on ten individuals of young, workers of *Apis mellifera* per replication were topically treated with imidacloprid dose ranged between 0.005 to 0.12 µg/bee. Mortality of bees was data recorded after 24 hours of treatments and subjected to probit analysis. LD₅₀ value worked out to be 0.037 µg/bee with a fiducial limit of 0.019 and 0.068 µg/bee. It was concluded that mortality data in relation to dose was homogeneous, as the determination of acute contact toxicity of imidacloprid is essential at sublethal levels.

Keywords: *Apis mellifera*, Imidacloprid, Neonicotinoid, LD₅₀ and Probit analysis

Introduction

The place of bees in the environment has many aspects; agronomic, economic, ecological and scientific. It plays an important economic role as a carrier of beekeeping (honey, royal jelly, pollen, propolis and wax) and agriculture by providing a quantitative and qualitative increase in crop production (Vaissiere 2002; Haubruge *et al.*, 2006; Breeze *et al.*, 2011) [15, 8, 3]. In addition, an ecological point of view, this is a useful insect bio- indicator of high environmental sensitivity because it is in contact with pollutants from various sources (Kevan, 1999) [10]. The phenomena of weakening of apiaries with a decrease in activity without the observation of pathogens (Faucon and Colin, 1983) [7]. This may be due to alterations in the nervous system of bees, especially since 90% of the insecticides used in agricultural and forest areas have neurotoxic properties. Imidacloprid is a systemic chloronicotinyl pesticide, belonging to the class of neonicotinoid insecticides. It works by interfering with the transmission of nerve impulses in insects by binding irreversibly to specific insect nicotinic acetylcholine receptors. The study of pesticides effects permits a correct evaluation of the risk posed by a certain product on honey bees. The effects provoked by a substance represent the result of the intrinsic toxic characteristic of the active ingredient and the administered dose or concentration. The intrinsic toxicity is a well know feature of all active molecules used as pesticides. Systemic insecticides represent at the same time the most effective type of plant protection products and one of the most important threat to honey bees as they contaminate essential food sources for pollinating insects. Concerning insecticides, the acute toxicity of active ingredients has been augmenting together with the introduction of new molecules; the most recently released products, neonicotinoids and phenyl pyrazoles, in fact, exhibit a significantly higher toxicity compared to all the other chemical classes (Casida, 2011) [4]. This fact contributes to give evidence to the hazard posed by neonicotinoids to honey bees, since very small doses may involve a considerable effect on mortality. The acute toxicity of insecticides allows the determination of a sublethal level (Chahbar *et al.*, 2014) [5]. This sublethal level is important to study the chronic toxicity of insecticides and their adverse sublethal effects that can induce a deleterious impact on honeybee populations.

Materials and Methods**Materials**

Technical grade of imidacloprid with 99.9% purity was procured from Sigma. To evaluate the contact toxicity of imidacloprid, young (two days of emergence), adult worker honeybees of *Apis mellifera* of similar age from adequately fed, healthy, as far as possible disease-free and

queen-right colonies with known history and physiological status were collected. During the morning hours, collection of young, adult worker honeybees of *A. mellifera* was done and starved for two hours before subjecting to the treatment. Before treatment the honeybees were immobilized by chilling for 2 minute in a refrigerator (4°C). Moribund honeybees was rejected and replaced by healthy honeybees before starting the test.

Modes of Treatment

Contact Application: The immobilized honeybees were individually treated with different doses (0.005, 0.01, 0.02, 0.04, 0.08 and 0.12 µg/bee) by topical application. A volume of 1 µl of solution containing the test substance was applied with the help of hand operated micro-applicator to the dorsal side of the thorax of each bee. A preliminary trial was run to adjust the range of doses which could give mortality between 20 to 80 percent. A complete test, finally comprised of three replications (10 worker honeybees/replication) of six different doses while the control honeybees were topically applied with the acetone only. For each replicate 10 treated worker honeybees were kept in a wooden cage (13 × 6 × 11 cm) in an incubator at 25±2 °C and 65± 5 percent relative humidity (EPPO, 1992). Treated honeybees were provided with 50% sugar syrup as food. Mortality was recorded after 24 hours of exposure. Honeybees which were unable to move or had uncoordinated movement were considered as dead.

Dose-mortality relationships and estimation of LD₅₀

Six doses of each insecticide which resulted in the adult worker bee mortality in the range of 20 to 80 per cent or close to this range was selected for calculation of LD₅₀ values. The mortality due to each insecticide was corrected using Abbott's correction (Abbott, 1925) [1]. Corrected mortality data were then subjected to probit analysis (Finney, 1971) to calculate LD₅₀ values.

Result and Discussion

The data on mortality of young, adult worker bees of *A. mellifera* when subjected to different doses of imidacloprid have been summarized in the Table 1. Under treatment and control mortality was recorded after 24 hours of exposure. The maximum mortality of 84.62% was recorded with a dose of 0.12µg/bee. The mortality of the young, adult worker bees of *A. mellifera* exceeding 50 percent was recorded with the doses between 0.04 and 0.08µg/bee whereas, at the doses of 0.005, 0.01 and 0.02 µg/bee with an exposure period of 24 hours resulted in 19.23, 23.07 and 26.92% mortality, respectively. Probit analysis of the data revealed that 0.037 µg was required to kill 50 per cent of the test population with a fiducial limit of 0.019 and 0.068 µg/bee (Table 1). Chi-square test showed that the data were homogeneous ($\chi^2_{cal} = 0.854$; $\chi^2_{tab} = 11.10$) at $p = 0.05$ and 5 degrees of freedom and probit kill followed linear relationship with log concentration as $Y = 1.266x + 3.02$. The present study are more or less in line with findings of Iwasa *et al.* (2004) [9] who reported the acute contact LD₅₀ (18ng/bee) of imidacloprid under laboratory conditions after 24 hours of exposure whereas Stark *et al.* (1995) [13] reported LD₅₀ (6.7–23.8 ng/bee) of imidacloprid under laboratory conditions. Suchail *et al.* (2000) [14] also reported the contact acute LD₅₀ (0.024 µg a.i./bee) of imidacloprid. However, substantial differences was reported as when the results of toxicity tests on honey bees performed by different laboratories are compared (Aupinel *et al.*, 2009; Blacquière *et al.*, 2012; Simon *et al.*, 2012) [2, 17, 16, 18] and when different honey bee subspecies or even colonies of a single subspecies are tested in the same laboratory with the same methodology (Suchail *et al.*, 2000; Laurino *et al.*, 2013) [14]. The differences in the toxicity to honeybee population might be due to differences in the genetic responses (Laurino *et al.*, 2013).

Table 1: Contact dose mortality response of *Apis mellifera* adults to imidacloprid

Dose (µg) × 1000	Log dose (X)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit (Y)	Expected probit
0.12	2.08	30	86.67	84.62	6.02	5.65
0.08	1.90	30	63.33	57.69	5.19	5.42
0.04	1.60	30	56.67	50.00	5.00	5.04
0.02	1.30	30	36.67	26.92	4.38	4.66
0.01	1.00	30	33.33	23.08	4.26	4.28
0.005	0.70	30	30.00	19.23	4.13	3.90
0.00	0.00	30	13	-	-	-

Regression equation: $Y = 1.266x + 3.02$

Heterogeneity:

χ^2_{Cal} = 0.854

χ^2_{Tab} = 11.10

LD₅₀ (µg) = 0.037

Fiducial limits (µg) = 0.019 and 0.068

Conclusion

On the basis of present studies the LD₅₀ value of imidacloprid to honeybee is 0.037 a.i. µg/bee and found that imidacloprid is highly toxic, thus should be applied in a restricted manner particularly to the crops where the honeybee are the main pollinating agents.

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