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Acute contact toxicity of neonicotinoids against Indian bees

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Abstract
Studies were conducted to assess the acute contact toxicity of neonicotinoids against Indian bees at Department of Agricultural Entomology, Tamil Nadu Agricultural University, India. Imidacloprid, acetamiprod, thiacloprid, thiamethoxam, clothianidin and dinotefuran were used for the study. Bioassay experiments were carried out to determine the median lethal concentration (LC_{50}) values and safety indices for *A. cerana indica*. The results reveal the LC_{50} values for the tested neonicotinoids were 32.26, 54.27, 96.86, 30.61, 40.31 and 103.28 ppm and the safety indices calculated on the basis of recommended spray concentration against the LC_{50} value were 0.81, 1.36, 1.61, 0.61, 1.01 and 2.07 respectively, for imidacloprid, acetamiprid, thiacloprid, thiamethoxam, clothianidin and dinotefuran. According to the safety index, thiamethoxam, followed by imidacloprid, are the least safe to bees.

Keywords: Honeybee, safety, median lethal concentration, neonicotinoids, safety index

Introduction
Pollinators provide an essential ecosystem service to both natural and agricultural ecosystems. Growing evidences suggests that healthy pollination services are threatened in many parts of the world. Pollinators ensure reproduction, fruit set development and dispersal in the vast majority plants. In turn, plants provide food and nesting sources for the pollinators. Among the pollinators, honey bees play an important role in pollinating the flowers, Bees and flowering plants are interdependent forming a mutually compatible system. Bees are the best pollen carriers and play a vital role in ensuring the productivity of crops. *Apis cerana indica*, the Indian honey bee, is one of the predominant bees found and domesticated in India, Pakistan, Nepal, Myanmar, Bangladesh, Sri Lanka, Thailand and mainland Asia. Relatively non-aggressive and rarely exhibiting swarming behavior, it is ideal for beekeeping. Several factors make this species efficient pollinators, the first being their smaller foraging range. The smaller colony size is also advantageous, as it makes them easy to transport and manage. Hence, the contribution of this species to the productivity of the cross pollinated crops is enormous. But at present pesticides have become synonymous with modern agriculture. Besides increasing agricultural production, they cause undesirable environmental effects including the effect on non-target species, such as honeybees and other pollinators.

Pesticides present the only group of chemicals that are purposely applied to the environment with aim to suppress plant and animal pests and to protect agricultural and industrial products. Currently, a variety of insecticides belonging to different classes are available for pest control, including pyrethroids, organophosphates, carbamates, neonicotinoids, botanicals and other novel insecticides of different origin that specifically act on insect metabolism and regulation of growth and reproduction [1]. Pesticides benefit the crops; however, they also impose a serious negative impact on the environment. Excessive use of pesticides may lead to the destruction of biodiversity. Neonicotinoids are a class of neuro-active insecticides chemically similar to nicotine. It includes acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam. This group dominated in the pesticide market for the management of sucking pest in agricultural and horticultural crops. Most of the crops grown in India, receive atleast one dose of the neonicotinoid spray before harvest. Recent studies revealed that, this group especially imidacloprid, acetamiprid and thiamethoxam cause detrimental to the survival, longevity and behaviour of honey bees [2-4]. Pesticides are a concern for sustainability of environment and global stability [5] so the safety of poisonous agro chemicals must be ensured. Hence present study was undertaken to study the acute contact toxicity of neonicotinoids against Indian bees.

Materials and Methods

Apis cerana indica was used for the bioassay studies. Honey bees were collected from hives maintained in the Insectary of Department of Agricultural Entomology, Tamil Nadu Agricultural University, India (11.016º N latitude and 76.929º E longitude). The colonies are maintained free from any pest and diseases and hive treatment with chemicals were not given before collecting the bees for the study. Adult worker bees were collected from the frame that contained honey and pollen (with the exception of the brood frame in order to avoid nurse bees) during morning hours. The frame with bees were taken and shaken over the muslin cloth bag (90×60 cm), coved the entrance with rubber band and transported to the laboratory immediately. Newly emerged workers were discarded. The bees were preconditioned for 2 hours and kept at 4ºC for 5 min for to facilitate easy handling.

To evaluate direct contact toxicity, various concentrations of formulated insecticides were prepared using water as solvent. The toxicants included in the study were: imidacloprid (Confidor 17.8 SL), acetamiprod (Manik 20 SP), thiacloprid (Alanto 21.7 SC), thiamethoxam (Actara 25 WG), clothianidin (Dantotsu 50 WDG) and Dinotefuran (Token 20 SG). All of the insecticides were examined at six concentrations (10, 20, 40, 80, 160 and 320 ppm) to obtain mortality in the range of 20 - 80 %. One treatment with water served as the untreated control. There were three replicates with ten bees each. The plastic boxes (10 cm diameter × 30 cm height) used for the experiment. Lid of the box having a 5 cm circular hole covered with plastic mesh in the middle to facilitate the aeration for honey bees. Circular filter paper (10 cm diameter) was used as surface of contamination for the contact toxicity assay.

During the experiments for determining lethal concentrations, the filter papers were taken and 1 mL solution of the desired concentration of insecticide was applied with a Potter spray tower. After treatment, filter paper was air dried for 10 minutes and kept inside the plastic boxes. Treatment details were marked on the plastic box. A batch of 10 chilled bees were taken and transferred to treatment box and closed with perforated lid and kept for observation. After one hour after exposure of honeybees to the respective concentration of insecticide the filter paper (contaminated surface) was removed from the box and honey bee is fed with absorbent cotton soaked with 50% sugar solution (w/v) given on the top of the mesh in the lid. Filter paper sprayed with distilled water was served as control. Experiment was conducted and maintained at 26±1 °C and 70% relative humidity. The number of dead or moribund test bees was counted at 24 h post exposure. Data on the mortality of test bees was converted into percentage mortality and corrected by Abbott's formula [6]. The values of Median Lethal Dose (LC_{50}) for different insecticides applied to test bees were calculated by probit analysis [7]. The safety index of different insecticides was calculated by following formula [8].

$$\text{Safety Index (SI)} = \frac{LC_{50} \text{ of insecticide (\%)}}{\text{NRC (\%)}}$$

Where NRC = Normal Recommended insecticide Concentration for crop pest control (%).

Results and Discussion

Bioassay experiments were carried out to determine the median lethal concentration (LC_{50}) values and safety indices for *A. cerana indica*. The mortality data obtained for series of concentrations of each insecticide recorded at 24 h were subjected to probit analysis to determine LC_{50} values. The LC_{50} values with fiducial limit for the tested insecticides are presented in Table 1. Contact dose mortality response curve of Indian bees to neonicotinoids were given in figure 1. The LC_{50} values for the tested neonicotinoids were 32.26, 54.27, 96.86, 30.61, 40.31 and 103.28 ppm for imidacloprid, acetamiprid, thiacloprid, thiamethoxam, clothianidin and dinotefuran, respectively. The calculated regression lines indicate a homogeneous response ($\chi 2>0.05$) of the *A. cerana indica* population to insecticides. The LC_{99} values also calculated and fudicial limts also given. Honey bees are valuable pollinators of cultivated crop plants and knowledge of the relative safety of insecticides during flowering is necessary to obtain maximum benefit from bee pollination. The safety index of each tested pesticide was calculated on the basis of recommended spray concentration against the LC_{50} value (Table 1). The safety index values were, 0.81, 1.36, 1.61, 0.61, 1.01 and 2.07 respectively for imidacloprid, acetamiprid, thiacloprid, thiamethoxam, clothianidin and dinotefuran. According to the safety index, thiamethoxam, followed by imidacloprid, are the least safe to bees. The results of the present study is in accordance with the reports of Khan and Dethe [9], who reported that the LC_{50} value obtained in *Apis cerana indica* for imidacloprid was 0.0035%. Pastagia and Patel [10] obtained 80.67% mortality of the Indian honey bee, *A. cerana*, after application of 0.05% imidacloprid.

Costa *et al.* [11] determined the topical LD_{50} for *Melipona scutellaris* was 2.41 ng/bee for 24 h and 1.29 ng/bee for 48 h. The oral LC_{50} for *M. scutellaris* was 2.01 ng .a. i./µL for 24 h and 0.81 ng a.i./ µL for 48 h. Imidacloprid was highly toxic to the honeybees as well as wild bees [12-15]. All these reports lend support to the present finding. Several researchers have reported differences in selectivity among the toxicants. The observed selectivity may be predicted on the basis of physiological, biochemical and behavioral differences between bee species and races. Besides genetic differences, experimental events such as the method of application, period of exposures, formulations and laboratory conditions are often responsible for the uneven responses of honey bees to pesticides [16].

Table 1: Direct contact toxicity of different insecticides to *A. cerana indica*

S. No.	Insecticide	Regression equation*	χ^2 value	LC_{50} (ppm)	Fiducial limits (LL - UP) (ppm)	LC_{99} (ppm)	Fiducial limits (LL - UP) (ppm)	Safety Index
1.	Imidacloprid	Y= 3.23+ 1.17 X	0.5154	32.26	21.29 - 48.87	820.97	285.44 - 2361.31	0.81
2.	Acetamiprid	Y= 3.41+ 0.92 X	0.1254	54.27	33.63 - 87.58	3370.33	574.42 - 19774.80	1.36
3.	Thiacloprid	Y= 3.15+ 0.94 X	0.1351	96.86	58.18 - 161.27	5580.81	816.92 - 38125.36	1.61
4.	Thiamethoxam	Y= 3.60+ 0.94 X	0.7434	30.61	18.23 - 51.39	1700.75	367.55 - 7869.70	0.61
5.	Clothianidin	Y= 3.65+ 0.84 X	0.4610	40.31	23.61 - 68.83	3574.90	503.19 - 25397.87	1.01
6.	Dinotefuran	Y= 3.21+ 0.89 X	0.3729	103.28	59.99 - 177.82	7323.14	880.66 - 60895.34	2.07

LC_{50} - Median lethal concentration; LL – Lower limit; UL – Upper limit; *y = a+ bx, where y is probit mortality, x is concentration of insecticides expressed as ppm solutions, a and b are regression coefficients.

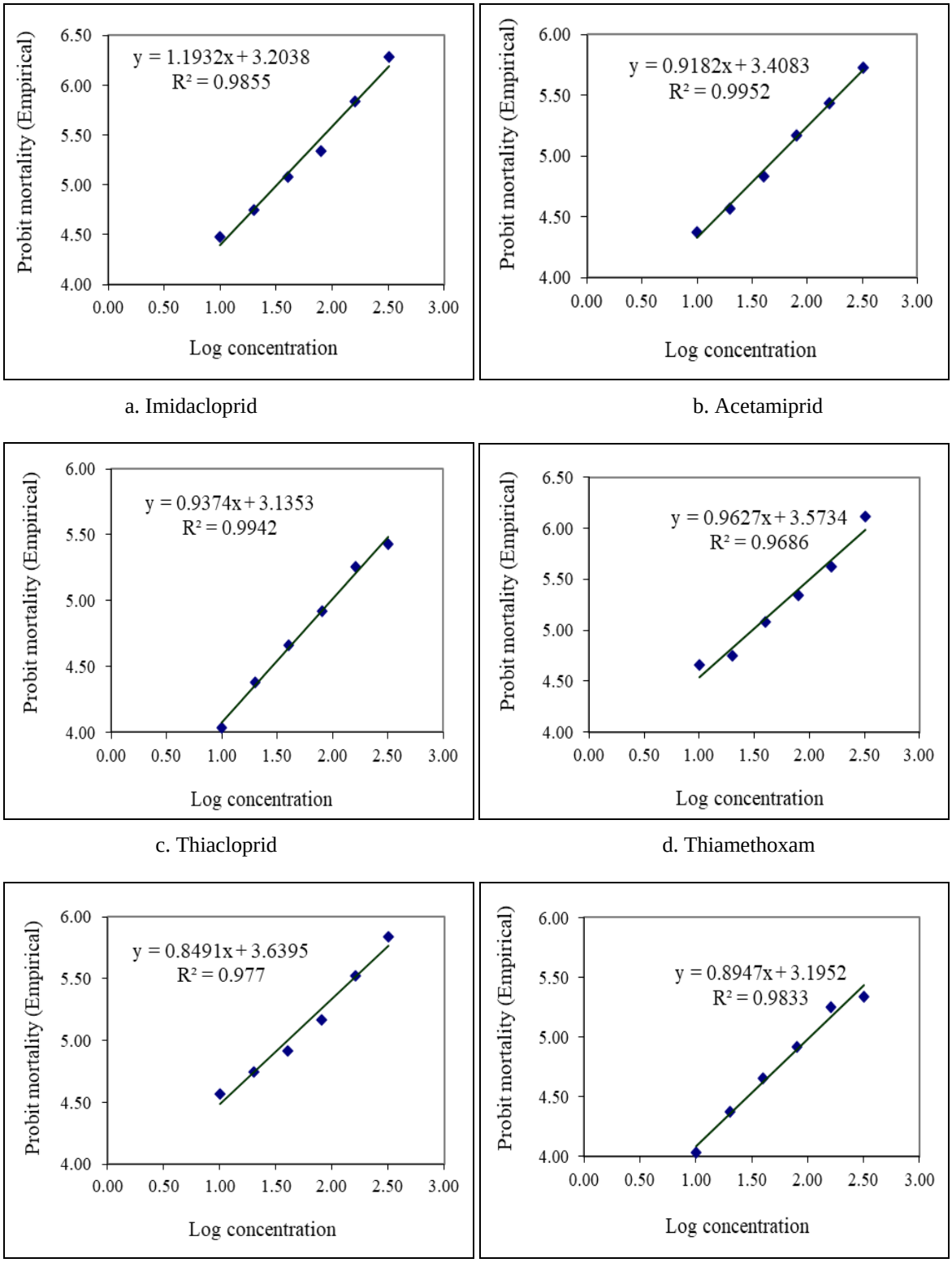

Fig 1: Contact dose mortality response curve of Indian bees to neonicotinoids

Conclusion

Among the neonicotinoids tested, imidacloprid and thiamethoxam recorded least LC_{50} values and safety index compared to acetamiprid, thiacloprid, clothianidin and dinotefuran. Hence, imidacloprid and thiamethoxam is the least safe to bees.

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