

Variable susceptibility of honeybee species *Trigona irridipennis* (smith) and *Apis mellifera* (linn.) to Dimethoate 30%EC

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ABSTRACT

In order to assess the toxicity difference between two honeybee species, a laboratory assay was conducted through oral exposure with, Dimethoate 30% EC to the common honeybee species, *Apis mellifera* and the stingless bee *Trigona irridipennis* as per OECD guideline. Different concentrations of the test product were prepared in 50% sucrose solution and provided to the bees with a dosing volume of 200µL/10 bees. After a maximum period of 6 hours, the test diets were replaced with sucrose solution (50%). Based on the weight difference of the feeder unit before and after exposure, the amount of treated diet /group of 10 bees were calculated and expressed as µg dose (dimethoate 30% EC) per bee. Observations for toxic signs and mortality were observed at 24 and 48 hours after dosing. The results of the study indicated that Dimethoate 30% EC recorded LD₅₀ of 0.020 and 0.017µg /bee to *Trigona irridipennis*, at 24 and 48 hrs after dosing, respectively. Similarly, LD_{95-24h} and 48h were observed to be 0.059 and 0.051 µg /bee, respectively. The LD₅₀ for *Apis mellifera* was observed as 0.321 and 0.304µg/bee at 24 and 48 hrs after treatment, respectively and LD₉₅ was observed as 0.408 and 0.434µg/bee at 24 and 48 hrs after treatment. The test item is about twelvefold more toxic to *Trigona irridipennis* than *Apis mellifera*. The lower LD₅₀ recorded by *Trigona irridipennis* results revealed that the sensitivity of the species to the insecticide when compared to *Apis mellifera* which is a globally recommended species for bee toxicity at laboratory.

Keywords: Dimethoate 30% EC, Honeybee toxicity, *Trigona irridipennis*, *Apis mellifera*, LD₅₀, LD₉₅,

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INTRODUCTION

Honey bees owing to its pollinating activity cater about one-third of the world's food supply. More than 50 major crops depend on these insects for pollination. Among the various bee species, stingless bees to which several medical uses are attributed apart from being effective pollinator are known for its valuable bee product with a long consumption tradition. Conversely, the bees become victimized to the plant protection chemicals which are unavoidable for the crop protection. The route of exposure of pesticides to the honeybee workers being oral and contact and the effect of the insecticides varies with the bee species. The honeybee *Apis mellifera* is valuable for the economy due to its hive by-products (honey, pollen, royal jelly) which generate considerable income for beekeepers. Honeybees also contribute to plant biodiversity by pollinating wild plants. Honeybees and their products are potentially exposed to several contaminants present in the environment, such as chemical products released into the hive to fight diseases and parasites, and pesticides used in agriculture against pests [1].

The stingless bee has size smaller to the honey bee *Apis mellifera* L., 1758 and its colonies are maintained in “meliponaries”, that are similar to apiaries. The hive of *Trigona irridipennis* is comprised of comb shaped overlapping disks surrounded by pots of food and its managed is similar to that of the honey bee. Besides its ecological importance as pollinators of native plants in India, *T.irridipennis* is considered a promising pollinator species for rearing on a large scale, to use in protected or field crops, due its ease of maintaining strong hives, which can be easily transported and multiplied. As trace amounts of Dimethoate 30% EC may be present in the

pollen and nectar of the treated plants, the intoxication of bees through feeding is one possibility. Thus, the aim of this work were to determine the variable susceptibility of lethal concentration (LD₅₀ and LD₉₅ at 24 h and 48 h) of the insecticide Dimethoate 30%EC for *Trigona irridipennis* foragers and to assess whether the *Trigona irridipennis* honey bee is a good model for toxicological studies, considering the diversity of Indian stingless bees. However, due to the accessibility of crops or the proximity of crops to native field areas, *T.irridioennis* is vulnerable to anthropic actions. One cause of increased mortality rates of these bees is insecticide poisoning [2]. This stingless bee has size similar to the honey bee *A. mellifera* L., 1758 and its colonies are maintained in the *A. mellifera* honey bee is a good model for toxicological studies, considering the diversity of Indian stingless bees. Hence knowledge and understanding of toxicity of the commonly used plant protection chemicals on different medically important bee species is essential which may help us to alter the usage pattern of insecticides in the field.

MATERIALS AND METHODS

The oral toxicity study was performed as described in the European and Mediterranean Plant Protection Organization [EPPO 1998) and The Organization for Economic Cooperation and Development (OECD 1998) Guidelines for the Testing of Chemicals, Honey bees, Acute Oral Toxicity Test (OECD 213 1998), forager adult worker bees of the same species were used for oral toxicity. Honeybees were obtained from adequately fed, healthy, disease-free, and queen-right colonies. Treated honeybees were held in metal cages with the size of 10 cm x 15cm x 8cm (length x width x height). Removable glass sheet in front side and perforated with ventilation holes were used in parts of the cage. Treatment doses were prepared by using sucrose solution in water (50% w/v). The honeybees were starved for up to 2 hours before the initiation of the test. Three replications (cages) were used in each treatment and the control. In each cage, 10 worker honeybees were transferred from hive by creating smoke using coconut coir. Before main test, arrange finding test was conducted with three concentrations (0.078, 0.108 and 0.128) for *Apis mellifera* and five concentrations (0.005, 0.012, 0.015, 0.033 and 0.045) for *Trigona irridipennis* using Dimethoate 30%EC and control (50% sucrose solution). Based on the range finding test, dosages for main experiment was fixed as 0.220, 0.304 and 0.361 µg /bee for *Apis mellifera* and 0.013, 0.035, 0.042, 0.093 and 0.128 µg /bee for *T. Irridipennis*. Since mortality of honeybees did not exceeded more than 10% between doses, the experiments were not prolonged. After a 6 hour of dosing the feeder unit were removed from the cages and post weighed. All honeybees were fed with sucrose solution in water (50% w/v), *ad libitum*. Honeybee mortality was recorded at 24, and 48h after the start of the test. The tests were performed in a dark room at a temperature of 25±2° C and 55 - 65% relative humidity [3-5]. Probit analysis was used to calculate the LD₅₀ and LD₉₅ of the test item using statistical package SAS 9.3.

RESULTS AND DISCUSSION

The results of the Dimethoate 30%EC indicated that the Dimethoate 30%EC doses viz., 0.078, 0.304 and 0.361 µg/bee recorded mortalities of 0, 37.7% at 24h and 7, 47.8% at 48h after treatment respectively for *Apis mellifera*. Whereas in *T. irridipennis* the doses 0.013 µg/bee and 0.035 µg /bee recorded 36.7% and 66.7% at 24 hrs and 46.7% and 80.0% at 48 hrs. At 24hrs, 93.3% and 100% mortality were observed at the maximum doses of 0.042 and 0.093 µg /bee respectively Figure 1 & 2. The LD₅₀ of Dimethoate 30% EC at 24 and 48 h after treatment to *T. irridipennis* was 0.019 and 0.016 µg/bee, respectively. Similarly, the LD₉₅ was calculated as 0.059 and 0.052 µg/bee at 24 and 48 h after treatment, respectively. The LD₅₀ of Dimethoate 30% EC to *A.mellifera* bees was 0.322 and 0.305 µg

/bee at 24 and 48 h of treatment, respectively. The LD₉₅ was observed as 0.408 and 0.433 μg /bee at 24 and 48 h after treatment, respectively. The results showing that *T. Irridipennis* are more sensitive to Dimethoate 30% EC than the Africanized honey bees *A. mellifera* Table 1 & 2.

This difference in the responses of various bee species to insecticide exposure was previously described by [6]. Changes in pesticide susceptibility among bee species were also observed by several authors [7]. Most of the results indicating that the honey bee *A. mellifera* was more tolerant to insecticides in comparison with species of stingless bees.

Figure 1 Comparison of Acute oral toxicity of Dimethoate 30% EC to *A. mellifera* and *T. irridipennis*

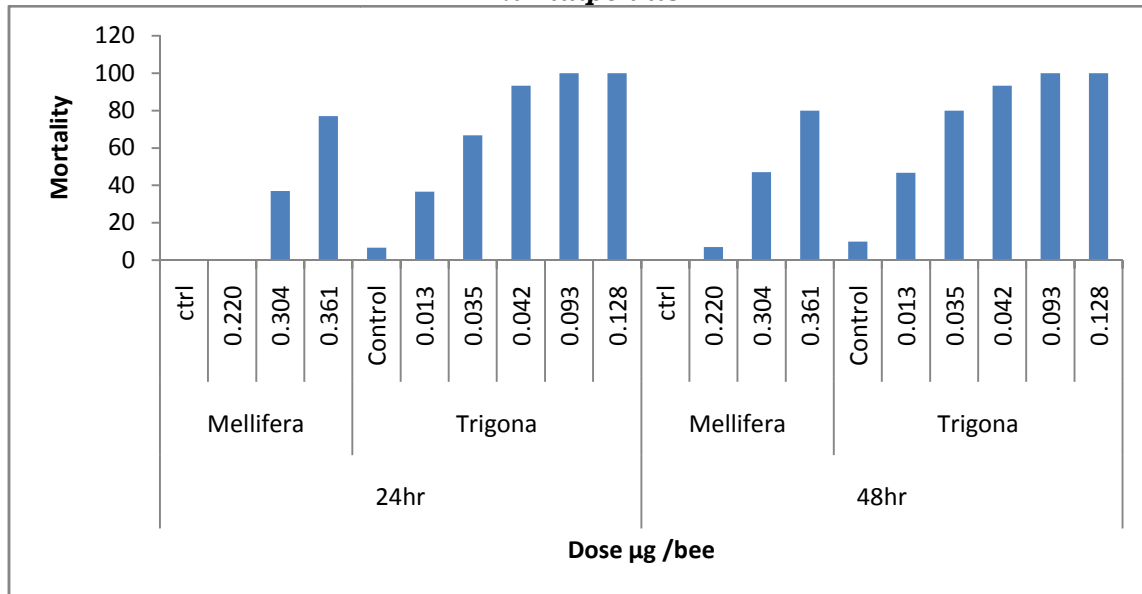
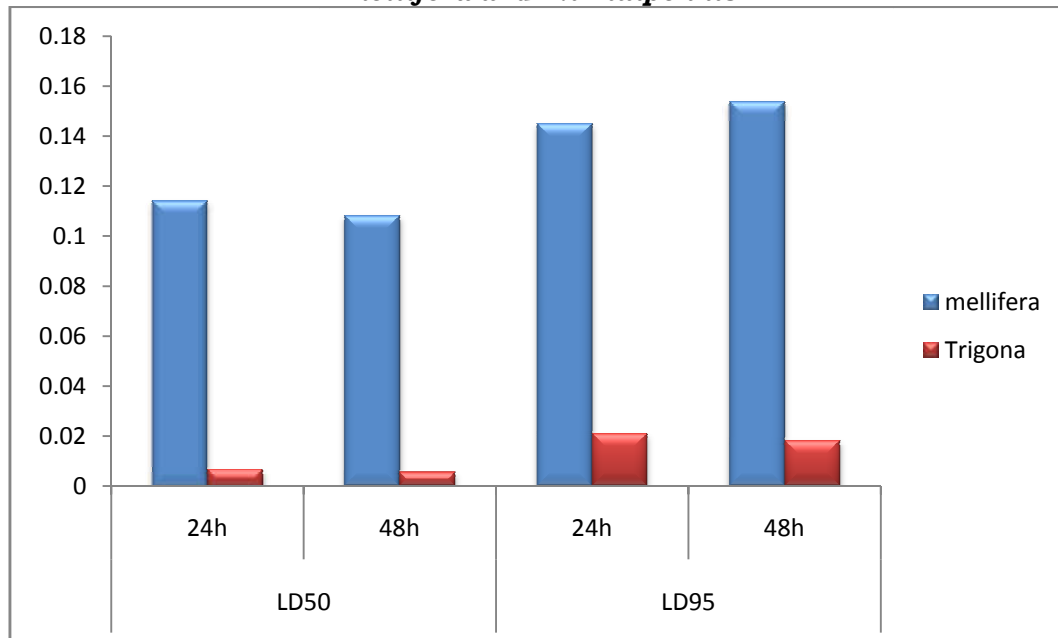


Figure 2 Comparison of oral LD₅₀ and LD₉₅ of Dimethoate 30% EC to foragers to *A. mellifera* and *T. irridipennis*



Furthermore, studies with the *A. mellifera* honey bee showed that sublethal doses of Dimethoate 30% EC can also be of concern because changes in behavior, such as in feeding and foraging, can affect the entire colony [8]. These findings reinforced the notion that wild bees are a pollinating group at high risk for pesticide exposure and toxicity [9]. The highest

concentrations of Dimethoate 30% EC used in this work (0.304 and 0.361 μ g a.i./bee based on consumption) resulted in mortality rates of 37.0 % and 77.0 %, respectively, following 24 h of exposure. After 48 h, at the highest concentrations of Dimethoate 30% EC resulted in 80 % mortality. Where as in *T. irridipennis* 0.042 μ g /bee and 0.093 μ g /bee 93.3% and 100% mortality after 48hrs in both the concentrations observed 100% mortality. When the lethal or behavioral effects of insecticides are replicated in bees under laboratory conditions, the greater impact of the pesticide under natural conditions is highlighted. Thus, it is important to establish limits on pesticide use, considering the consequences on biodiversity, economic losses from beekeepers and crop producers, and the awareness of society concerning the pesticides in environmental [10]. The use of diverse pollinator species in toxicological studies allows a better understanding of the spectrum of bee responses. This is especially important when compared the results of non-*Apis* bee with those of the current model *A. mellifera* [9]. In this way, new studies on *T. irridipennis* behavior contamination with sublethal doses of Dimethoate 30% EC are being conducted. It is concluded from the present study that the non-*Apis* species, *T. irridipennis* is more sensitive to xenobiotic than the common honeybee species, *Apis mellifera*. Hence, the stingless bee, *T. irridipennis* can also be used as a good model for ecotoxicological studies.

Table 1: Toxicity effect of Dimethoate (30% EC) on *Apis mellifera* and *Trigona irridipennis* after 24 hrs treatment

| Species | Dose | % of Mortality* (mg/L) \pm SE | LC ₅₀ \pm SE (mg/L) | UCL-LCL (mg/L) | LC ₉₅ \pm SE (mg/L) | UCL-LCL (mg/L) | χ^2 (df=1) |
|--|---------|---------------------------------|----------------------------------|-----------------|----------------------------------|-----------------|-----------------|
| <i>Apis mellifera</i> (Control) | Control | 0.0 \pm 0.0 | 0.114 | 0.121 - 0.108 | 0.145 | 0.178 - 0.134 | 0.6404 |
| | 0.078 | 0.0 \pm 0.0 | | | | | |
| | 0.108 | 36.7 \pm 8.8 | | | | | |
| | 0.128 | 76.7 \pm 3.3 | | | | | |
| <i>Trigona irridipennis</i> (Experiment) | Control | 6.7 \pm 6.7 | 0.0069 | 0.0101 - 0.0025 | 0.0211 | 0.1304 - 0.0134 | 0.0016 |
| | 0.005 | 36.7 \pm 3.3 | | | | | |
| | 0.012 | 66.7 \pm 14.5 | | | | | |
| | 0.015 | 93.3 \pm 6.7 | | | | | |
| | 0.033 | 100 \pm 0.0 | | | | | |
| | 0.045 | 100 \pm 0.0 | | | | | |

Table 2: Toxicity effect of Dimethoate (30% EC) on *Apis mellifera* and *Trigona irridipennis* after 48hrs treatment

| Species | Dose | % of Mortality* (mg/L) \pm SE | LC ₅₀ \pm SE (mg/L) | UCL-LCL (mg/L) | LC ₉₅ \pm SE (mg/L) | UCL-LCL (mg/L) | χ^2 (df=3) |
|--|---------|---------------------------------|----------------------------------|-----------------|----------------------------------|-----------------|-----------------|
| <i>Apis mellifera</i> (Control) | Control | 0.0 \pm 0.0 | 0.108 | 0.116 - 0.101 | 0.154 | 0.192 - 0.138 | 0.6952 |
| | 0.078 | 6.7 \pm 3.3 | | | | | |
| | 0.108 | 46.7 \pm 3.3 | | | | | |
| | 0.128 | 80.0 \pm 5.8 | | | | | |
| <i>Trigona irridipennis</i> (Experiment) | Control | 10.0 \pm 5.8 | 0.0056 | 0.0064 - 0.0046 | 0.0183 | 0.0230 - 0.0155 | 0.2481 |
| | 0.005 | 46.7 \pm 12.0 | | | | | |
| | 0.012 | 80.0 \pm 11.5 | | | | | |
| | 0.015 | 93.3 \pm 6.7 | | | | | |
| | 0.033 | 100 \pm 0.0 | | | | | |
| | 0.045 | 100 \pm 0.0 | | | | | |

Abbreviation: **Dose** – Based on actual consumption, **LC₅₀**:lethal concentration that kills 50% of the exposed larvae, **LC₉₅**:lethal concentration that kills 90% of the exposed larvae. **UCL**:upper confidence limit, **LCL**:lower confidence limit, **χ^2** : chi-square, **df**: degree of freedom.

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