

# Influence of Pesticide Dose Preparation on Toxicity of Honeybee Species, *Apis mellifera* L. by Contact Method

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**Abstract** The aim of this study was to compare the effect of dose prepared from two different stocks in an acute contact toxicity test to honeybees, *Apis mellifera* L. under laboratory condition. Dimethoate 30% EC contains an active ingredient of 90.18 % w/w which was used as the test substance. The test was conducted as per OECD Guideline 214 (1998). The test stock solution 69.92 ppm (Stock I) and 4.3ppm (Stock II) were prepared in deionized water. The toxicity was evaluated against honeybees at the doses viz., 0.045, 0.068, 0.101, 0.152 and 0.228 µg a.i./bee prepared from both stock solutions. Bees treated with Dimethoate 30% EC dissolved in deionized water were dosed by topical application on the dorsal side of thorax of each bee. The control (Deionized water) was maintained for comparison. Assessments of mortality and abnormal behavioural effects were carried out at 4, 24 and 48 hours after dosing. Dimethoate 30% EC (Stock I) at the highest tested nominal dose of 0.228 µg a.i./bee recorded the highest mortality of 70% and the lowest dose 0.045 µg a.i./bee recorded 0% mortality at 48 hours after dosing. Whereas dimethoate 30% EC (Stock II) at the highest tested nominal dose of 0.228 µg a.i./bee recorded the highest mortality of 23.33% and the lowest dose 0.045 µg a.i./bee recorded 0% mortality at 48 hours after dosing. The 48-hour Contact LD<sub>50</sub> (Stock I) was 0.160 µg (95% Confidence limits: 0.135 - 0.201) dimethoate/bee at 48 hours after dosing. The 48-hour Contact LD<sub>50</sub> (Stock II) was 0.319 µg (95% Confidence limits: 0.135 - 0.201) dimethoate/bee at 48 hours after dosing. All the control

groups (Deionized water) recorded 0.0% mortality during the assessment made up to 48 hours after dosing. Observations of sublethal (abnormal behavioural) effects included moribund, knockdown and apathy at 24 hours after dosing the test substance. No sub lethal effects were observed at 4 and 48 hours after dosing. The test met all the validity criteria as described in OECD 214.

**Keywords** *Apis mellifera*, OECD, Contact Toxicity, Honeybee, Dimethoate

## 1. Introduction

Honeybee, being a natural pollinator, is exposed to different types of pesticides sprayed in field condition both topically and by orally. Therefore, the toxicity is evaluated under laboratory condition both by contact exposure and oral feeding to assess toxic nature of the pesticides when honeybees are victimized. The western honeybee or European honeybee (*Apis mellifera*) is the most common of the 7-12 species of honeybees worldwide. It provides highly valued pollination services for a wide variety of agricultural crops [9] and ranks as the most frequent single species of pollinator for crops worldwide [5]. Compound and dose-dependent effects might represent respective chemical structural differences determining an observed effect, and thresholds of compound effects on honeybee

physiology [13].

The effect of oral octopamine (OA) on the bees responsiveness to sucrose was OA dose dependent and lasted for up to 1 hour in stingless bees (*Melipona scutellaris*) studied by Cabe [4]. Bartling [3] showed that the exposure to low doses of pesticides induces an immune response and the production of nitric oxide in honeybees. Piotr medrzycki [1,12] studied that sub-lethal doses alter the honeybee behavior therefore the period of time in which honeybee behavior is altered, which could negatively affect both the individual and the entire colony. Combinational effects of pesticides on life cycle of honeybees and Model for quantifying pesticide field exposure and ecotoxicity effects of bees were demonstrated by Leonora crenna [8]. The Dose dependent effect of pesticides, combinational pesticide effect causing acute toxicity, behavior changes, alteration in social responses and auto immune responses were been well studied. The possible influence of preparation method of test dosage in the solvent is considered in the present study.

## 2. Materials and Methods

The objective of this study is to compare the acute effect of dose strength on toxicity of Honeybee species, *Apis mellifera* L and *Apis cerana indica* by contact exposure method. Dimethoate 30% EC was used as the test substance. The dose that causes 50% mortality (LD<sub>50</sub>) after 48h was determined. Abnormal behavioral effects were been monitored and reported. The test was conducted in the laboratory for a 48hour period following the OECD guideline No. 214 (1998) [11,12].

### 2.1. Collection of Bees

Young adult worker honeybees, *Apis mellifera* L, (Hymenoptera, Apidae) adequately fed, healthy, disease-free and queen-right bee hive, maintained at IIBAT. Bees were collected from the combs without brood during the morning hours directly in the cage, brought to the test laboratory and kept in humidity chamber. The cages with healthy bees were been selected for study. The bees were being starved in the test cages (starving period included within acclimation period) for about 2 hours before dosing. The time at which the dosing starts was considered as end of acclimation. After dosing, the cages containing the bees were being placed in the humidity chamber. Honeybees may come into contact with pesticide residues through oral exposure routes during the normal course of pesticide applications. The test system used in this study evaluates the potential

toxicity to honeybees based on contact exposure.

### 2.2. Test Cages and Test Conditions

The Stainless steel cages of Size 10 cm × 8.5 cm × 5.5 cm (length × height × width) covered at front Side with removable glass slide and at the bottom with wire mesh, perforated with ventilation holes. The test cages were being incubated at 25 ± 2 °C and 50 - 70 % RH.

### 2.3. Preparation of Test Doses

Nominal doses of the test substance tested were 0.045, 0.068, 0.101 0.152 and 0.228 µg a.i./bee. For the preparation of Stock, I, 0.06992 g of test substance was weighed out and volume was made up to 10 mL using deionized water (Stock - 1). Stock - 2 was prepared by adding 0.615 mL from stock - 1 made up to 10 mL using deionized water. The required doses were prepared by diluting the stock solution 1 and 2 using deionized water, such that the intended nominal dose for one bee was found in 1 µL (Table 1 and Table 2).

### 2.4. Application of Doses

Bees were being anesthetized using CO<sub>2</sub>. A single 1-µL droplet of the test solution (test substance group), reference solution (reference substance group) and control group (deionized water) was been applied on the dorsal surface of the bee thorax by using a Burkard - Micro applicator. After dosing, the cages containing the treated bees were provided with 50% w/v sucrose solution *ad libitum* using feeder unit and were placed in the humidity chamber.

### 2.5. Observations

30 bees were being used at each dose level of the control and the test substance groups (3 replicates per test unit; 10 bees per replicate test unit). Number of dead bees and behavioral abnormalities were being recorded at 4 ± 1 hour, 24 ± 1 and 48 ± 1 hours after dosing. If mortality is observed in any of the replications of control group, the mean treatment mortality will be corrected against control mortality using Abbott's formula [2]

### 2.6. Statistical Analysis

The 24 & 48 hour LD<sub>50</sub> value of Dimethoate 30% EC (Stock I & II) with 95% confidence intervals for test substance groups were been analysed by Probit analysis, NOEC between control and treatments were Analyzed by Mann-Whitney U Test using the statistical Software IBM SPSS Statistics Version 28 [7] and found to be statistically significant(P<0.05) Between control and treatment.

**Table 1.** Preparation and Calculation of Stock Solution I

Doses ( $\mu\text{g a.i./bee}$ )	Volume of stock ( $\mu\text{L}$ )	Final volume (mL)
0.045	18.2	1.0
0.068	27.5	1.0
0.101	40.7	1.0
0.152	61.2	1.0
0.228	91.8	1.0

0.06992 g of Dimethoate 30% EC in 10.0 mL using deionized water

**Table 2.** Preparation and Calculation of Stock Solution II

Doses ( $\mu\text{g a.i./bee}$ )	Volume of stock ( $\mu\text{L}$ )	Final volume (mL)
0.045	295.3	1.0
0.068	446.5	1.0
0.101	661.4	1.0
0.152	995.3	1.0
0.228	1492.95	1.0

0.615 mL of stock I in 10.0 mL using deionized water

### 3. Results and Discussions

The test met all the validity criteria, as mortality in the controls did not exceed 10% (All the controls recorded 0% mortality up to 48 hours after dosing) and the response in Dimethoate 30% EC exposure groups was within the specified range (0.10 to 0.30  $\mu\text{g}$  dimethoate/bee) as described in OECD 214.

No mortality was observed at the test dose of 0.045  $\mu\text{g}$  a.i./bee at 24 & 48 hours after dosing in both Stock I and Stock II. Maximum mortality of 16.67%, 63.33% and 70.0% was observed on 0.228  $\mu\text{g}$  a.i./bee at 4, 24h & 48h respectively of stock I. Maximum mortality of 16.67%, 16.67% and 23.33% was observed on 0.228  $\mu\text{g}$  a.i./bee at 4, 24h & 48h respectively of stock II. Minimum mortality of 16.67%, 16.67% and 17.0% was observed on 0.068  $\mu\text{g}$  a.i./bee at 4, 24h & 48h respectively of stock I. Minimum mortality of 6.66%, 6.66% and 13.33% was observed on 0.152  $\mu\text{g}$  a.i./bee at 4, 24h & 48h respectively of stock II. Both the control groups (Deionized water) recorded 0.0% mortality at the assessment made at 24 and 48 hours after dosing (Table 3).

No sub lethal effects were observed at 4 hours after dosing. In Stock I, 10.0 % bees were found apathetic, 70% lying on the back and 25.0% moribund in the doses of 0.101, 0.152 and 0.228  $\mu\text{g}$  a.i./bee at 24 hours after dosing. In Stock II, 25.0% and 5.0% were moribund in the doses of 0.152 and 0.228  $\mu\text{g}$  a.i./bee at 24 hours after dosing. No sub lethal effects were been observed at 48 hours after dosing. The 24-hour Contact  $\text{LD}_{50}$  (Stock I) was 0.184  $\mu\text{g}$  (95% Confidence limits: 0.153 - 0.248) dimethoate/bee at 48 hours after dosing (Table 4).

The 24-hour Contact  $\text{LD}_{50}$  (Stock II) was 0.371  $\mu\text{g}$

(95% Confidence limits: 0.262 – 6.351) dimethoate/bee at 24 hours after dosing. The 48 hour Contact  $\text{LD}_{50}$  (Stock I) was 0.160  $\mu\text{g}$  (95% Confidence limits: 0.135 - 0.201) dimethoate/bee at 48 hours after dosing. The 48-hour Contact  $\text{LD}_{50}$  (Stock II) was 0.319  $\mu\text{g}$  (95% Confidence limits: 0.135 - 0.201) dimethoate/bee at 48 hours after dosing. Though, the 24  $\text{LD}_{50}$  was within the range (0.1 - 0.3  $\mu\text{g}$  dimethoate/bee, OECD 237), the data resulted from the stock I & II was not comparable. The mortality data of Stock I and Stock II at each dose level was significantly different (a non-parametric Mann-Whitney T Test) (Figure 1).

Several studies were being done on the toxicity data generation [6] but there is less data on comparison of results to the doses achieved with different stock concentration. The deleterious effects on the development, behavior, physiology, and survival of honeybees due to acute and chronic exposures to sublethal doses of neonicotinoid insecticides, including imidacloprid and acetamiprid, have been widely documented in the study done by Steven [13]. Exposure to imidacloprid induces autophagy and apoptosis in the midgut of bees and was been studied by Lenise Silva Carneiro [8]. The study on honeybee colony activity and thermoregulation after treating with sublethal concentrations of methoxyfenozide was carried out by Meikle, W. G [14].

To our understanding, no studies have been carried out on the toxicity of Dimethoate to *Apis mellifera* at different test substances stock with the same doses. In our study, the major findings are that the dimethoate is more harmful to *Apis mellifera* at a higher stock (0.06992 ppm), compared to dose prepared at the lower stock (0.0043 ppm). We clearly show that stock concentration should be

considered when evaluating risk of pesticides to realistic exposure scenarios including mixtures of honeybees. Further study is needed to identify the effects of pesticides. of lower doses at various stock strength, as well as other

**Table 3.** Data on Mortality of Honeybees Treated with Dimethoate 30% EC

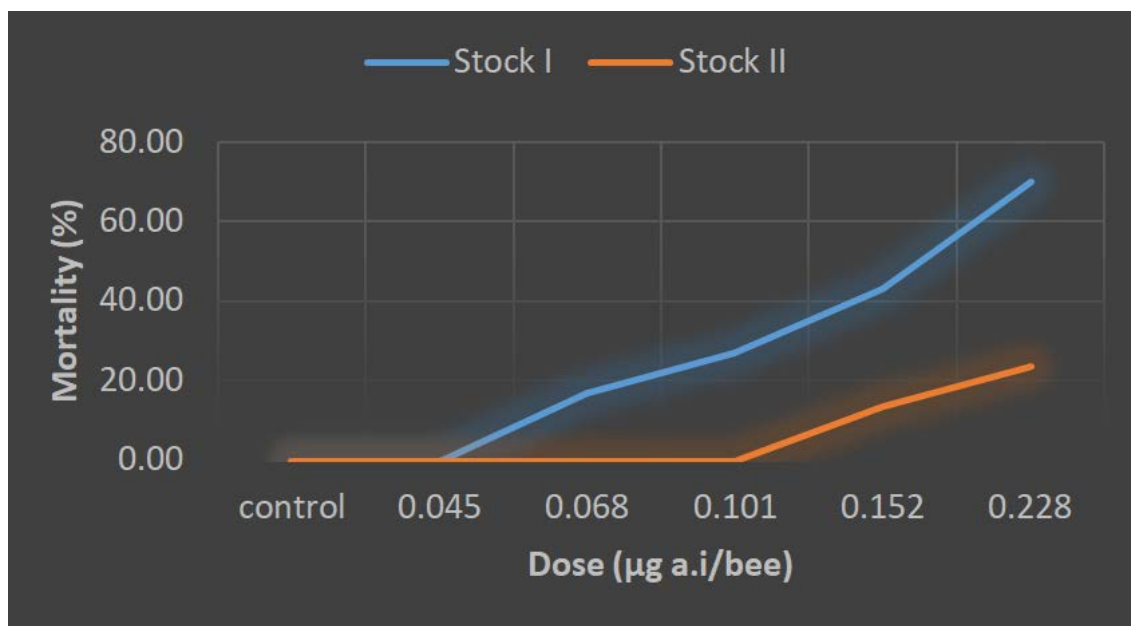
Dimethoate 30% EC ( $\mu\text{g a.i./bee}$ )	Stock I [%]a			Stock II [%]a		
	4h	24h	48h	4h	24h	48h
Control (Deionized water)	0.0	0.0	0.0	0.0	0.0	0.0
0.045	0.0	0.0	0.0	0.0	0.0	0.0
0.068	0.0	13.33 (5.77)	16.67 (5.77)	0.0	0.0	0.0
0.101	0.0	23.33 (5.77)	26.67 (11.55)	0.0	0.0	0.0
0.152	10 (0)	33.33 (15.28)	43.33 (23.09)	6.67 (5.77)	6.67 (5.77)	13.33 (5.77)
0.228	16.67 (5.77)	63.33 (15.28)	70.0 (20.0)	16.67 (5.77)	16.67 (5.77)	23.33 (5.77)

<sup>a</sup>Mean of three Replications; Figures in parenthesis are standard error

**Table 4.** Data on Behavioral abnormalities of Honeybees Treated with Dimethoate 30% EC

Dimethoate 30% EC ( $\mu\text{g a.i./bee}$ )	Stock I [%]a			Stock II [%]a		
	4h	24h	48h	4h	24h	48h
Control (Deionized water)	0.0	0.0	0.0	0.0	0.0	0.0
0.045	0.0	0.0	0.0	0.0	0.0	0.0
0.068	0.0	0.0	0.0	0.0	0.0	0.0
0.101	0.0	10.0 ap 15.0k	0.0	0.0	0.0	0.0
0.152	0.0	55.0 k	0.0	0.0	25.0 k	0.0
0.228	0.0	25.0m	0.0	0.0	5.0m	0.0

<sup>a</sup> Mean of three Replications; Figures in parenthesis are standard error. k –lying on the back; m - moribund (bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation, e.g., light, air movement, bees may recover but usually die); ap - apathy (bees show only slow or delayed reactions to stimulations (e.g., light, air movement); bees are either sitting motionless in the cage or staggering



**Figure 1.** A comparison on 48 hour mortality data (Stock I & II)

## 4. Conclusions

The effects of dimethoate 30% EC were assessed in contact honeybee toxicity test conducted in the laboratory. The 48-hour Contact LD<sub>50</sub> (Stock I) was 0.160 µg (95% Confidence limits: 0.135 - 0.201) dimethoate/bee at 48 hours after dosing. The 48-hour Contact LD<sub>50</sub> (Stock II) was 0.319 µg (95% Confidence limits: 0.135 - 0.201) dimethoate/bee at 48 hours after dosing. There is a significant difference observed on the mortality data among the doses achieved from the two different test substance stock solutions.

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