# Toxicity and Poisoning Symptoms of selected Insecticides to Honey Bees (*Apis mellifera mellifera* L.)

Vrushali Vijaykumar Pashte[1,*] and Chidanand Shivshankar Patil[2]

[1] *Department of Rural Technology and Social Development, School of Natural Resources*, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, Chhattisgarh (495009), India

[2] *Pesticide Residue Laboratory, Department of Entomology, Post Graduate Institute*, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra (413722), India

***Corresponding author**: pashte.vrushali@gmail.com

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**Abstract:** Bees are potential pollinators of wide variety of crops. The European dark bee, *Apis mellifera mellifera* (L.) is widely used for crop pollination. However, pesticide usage in modern agriculture has threatened the plant-bee pollinator interaction. There is lack of data regarding lethal time, insecticide concentration and poisoning symptoms, especially for formulated insecticides that are widely used in insect management. This study shows that the intrinsic toxicity of insecticides ($LC_{50}$) to *A. mellifera mellifera* (L.) was in the following order: imidacloprid (0.0070) > fipronil (0.0125) > indoxacarb (0.0266)> cypermethrin (0.0370) > dimethoate (0.0385). The lethal time ($LT_{50}$) values (h) in the ascending order of toxicity of insecticides were as follows: fipronil (6.56), cypermethrin (6.69), dimethoate (8.00), imidacloprid (9.85) and indoxacarb (13.45). Distinct poisoning symptoms observed in *A. mellifera mellifera* were extended proboscis, expanded wings, unhooked wings, extended legs and twisted bodies, defecation on cage covers, sting in release-out position and anus with excreta. All the tested pesticides are harmful to the honey bee except azadirachtin. The tested pesticides exhibited different poisoning symptoms in bees, which could be useful for beekeepers in identifying the cause of colony mortality. In conclusion, the pesticide toxicological research on bees is an important safety aspect for beneficial organisms. This study reveals a realistic acute toxicity in the field of commonly used insecticides. The information is important for insecticide selection in order to minimize direct killing of foraging honey bees while maintaining effective management of crop pests.

**Key words**: bioassay; intrinsic toxicity; $LC_{50}$; time mortality; *Apis mellifera mellifera* L.

## INTRODUCTION

Pollination by insects is an important ecosystem service because crop plants account for 35% of global crop-based food production benefitting from insect-mediated pollination [1]. Bees (Hymenoptera: Apiformes) are the primary pollinators for many crops, with modified morphological, anatomical makeup and behavioral characters [2,3]. Honey bees have been used commercially for qualitative and quantitative improvement of the production of various fruits, seeds, including oilseeds, nuts and fiber crops [3-5]. Bee pollination also improves crop shelf life and commercial value [6].Unfortunately, honeybee populations are in decline since the 1990s, possibly due to a combination of pests, diseases, poor diet, colony collapse disorder and the increasing use of different pesticides [7,8].To date, there is no single factor that can explain colony loss in bees; however, one anticipated factor is the extensive application of chemicals for crop management [9]. Bee poisoning from pesticides is a serious problem worldwide [7,9–11].

Pesticides are often considered an easy, quick and inexpensive solution for managing weeds and insect pests in agriculture and in urban landscapes. Pesticide contamination poses considerable risks to the surroundings and non-target organisms [12].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 [13]. The negative impacts of insecticides have been demonstrated for the honeybee [14-18] and some species of wild bees [19-21]. Several botanical insecticides, which are often considered as harmless and environmentally friendly, can generate acute toxicity and sublethal effects on honey bees [22].

Any chemical used for pest management should be studied carefully for its toxicity to non-target organisms. Earlier assessments of insecticide toxicity for honey bee have mostly been undertaken with technical grade insecticides [23,24]. Such tests cannot always provide farmers with sufficient information about formulated insecticides. However, in taking pest management decisions aimed at sustaining crop production by employing pesticides, bee safety must be ensured. Pest management must take into account a judicious management of pollinators. In the present investigation, we examined the toxic impact of different formulated insecticides (azadirachtin, dimethoate, cypermethrin, fipronil, imidacloprid and indoxacarb) on European dark bees (*Apis mellifera mellifera* L.) after direct topical exposure. We analyzed the survival and mortality time as well as the poisoning symptoms.

## MATERIALS AND METHODS

### Test organism

*Apis mellifera mellifera* (L.) was used for the bioassay studies. Honey bees were collected from hives maintained in the central campus, Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra State), India (19°20'47"N latitude and 74°38'47"E longitude). The hives were examined for the presence of diseases and pests during routine colony maintenance. Throughout the experiment the colonies were free from diseases and pests. Therefore, no hive treatment of any chemical was conducted prior to and during the studies.

### Collection and inactivation of bees

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 [25]. The bees were shaken from the frames into a large muslin cloth bag (90×60 cm). The opening of the bag was covered with a rubber band and the bees were transported immediately to the laboratory. Newly emerged workers with light yellow setae on the thorax were discarded [26]. The bees were preconditioned for 2h and anaesthetized by chilling for 5 min to facilitate easy handling. The chilling method was used with slight modifications as recommended by Thomas and Phadke [27] and Human et al. [28]. Before the start of the bioassay, the mortality and activation period were noted for different periods of exposure at low temperature (0-4°C). The bees were chilled for 5min to make them temporarily inactive.

### Preparation of toxicants

To evaluate direct contact toxicity, various concentrations of formulated insecticides were prepared using acetone as solvent. The toxicants included in the study were: azadirachtin (NEEMRAJ 0.15%), dimethoate (TATA TAFGOR®30% SC), cypermethrin (CYPER PLUS10% EC), fipronil (DEVIGENT PLUS™ 5% SC), imidacloprid (TRISHUL 17.8% SL) and indoxacarb (INDEX 14.5% SC). All of the insecticides were examined at six concentrations to obtain mortality in the range of 20-80%. One treatment with acetone served as the untreated control. There were four replicates with ten bees each.

### Experimental conditions

The cages used for the experiment were made of metal wire and had a cylindrical shape (40 cm height × 30 cm diameter) and were covered with a muslin bag that was open on the upper side to facilitate the release of bees. A 50% sugar solution (w/v) was given in 5-mL-plastic vials with screw caps. Before filling the tube, the bottom end was punctured with a fine needle and covered with a ball of medicated cotton. The treated bees in cages were maintained at 26±1°C and 70% relative humidity. The cages were protected from ants by marking with 'Krazy Lines Plus'.

### Laboratory bioassay with bees

During the experiments for determining lethal concentrations, after chilling, batches of ten bees were separated in a plastic Petriplate (10 cm in diameter)

that contained a sheet of filter paper. The 0.5 mL of solution of the desired concentration of insecticide was applied with a Potter spray tower. After treatment, the bees were transferred immediately to the specially designed cylindrical cage. The feeding tube (sugar solution) was hung in the cage before the treated bees were transferred to the cage. The injection unit of the Potter tower was rinsed thoroughly with acetone between each insecticide treatment. The number of dead or moribund test bees was counted at 24 h post exposure. For determination of the mortality time ($LT_{50}$), the collection, inactivation and application of insecticides was the same as in studies of lethal concentration.

To determine the $LT_{50}$, the recommended dose of each formulated insecticide [azadirachtin 0.15% (5ml/L), dimethoate 30% EC (200 g of active ingredient $ha^{-1}$), cypermethrin10% EC (65 g a.i. $ha^{-1}$), fipronil 5% SC (45 g a.i. $ha^{-1}$), imidacloprid 17.8% SL (20 g a.i. $ha^{-1}$) and indoxacarb 14.5% SC (44 g a.i. $ha^{-1}$)] were prepared using acetone as solvent. The 0.5-mL solution of the recommended concentration of insecticide was applied using the Potter spray tower. One treatment with acetone served as an untreated check. There were three replicates with ten bees each. After the treatment, the bees were immediately transferred to the cages. Bee mortality was examined at successive intervals after treatment: 1, 2, 4, 6, 8, 24 and 48 h

While determining the time of death, the treated bees were observed for specific symptoms of insecticide toxicity. Their feeding behavior and movements in the cages were also observed at successive intervals. The symptoms exhibited by dead worker bees were observed using a stereomicroscope.

**Data analysis**

Data on the mortality of test bees was converted into percentage mortality and corrected by Abbott's formula [29]. The values of $LC_{50}$ for different insecticides applied to test bees were calculated by probit analysis [30]. The safety index of different insecticides was calculated by the formula of Hameed et al. [31]:

$$S.\ I. = \frac{LC_{50}}{NRC}$$

Where, S. I. is the safety index, $LC_{50}$ the median lethal concentration of insecticide (%) and NRC the normal recommended insecticide concentration for crop pest control (%).

The data on cumulative mortality at various time intervals was corrected by Abbott's formula and used for calculation of $LT_{50}$ (median lethal time) values at the recommended dose of each formulated insecticide.

## RESULTS AND DISCUSSION

### Direct contact toxicity of different insecticides to *A. mellifera mellifera* (L.)

Bioassay experiments were carried out to determine the median lethal concentration ($LC_{50}$) values and safety indices for *A. mellifera mellifera*. 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. The $LC_{50}$ values in ascending order from the most to the least toxic insecticide for the worker bees were as

**Table 1.** Direct contact toxicity of different insecticides to *A. mellifera mellifera*

| Sr. No. | Insecticide | Regression equation | $\chi^{2*}$ | $LC_{50}$ (%) | Fiducial limits | | Relative toxicity | Safety index |
|---|---|---|---|---|---|---|---|---|
| | | | | | UL (%) | LL (%) | | |
| 1 | Dimethoate | y = 7.929005 + 2.070336 X | 5.2493 | 0.0385 | 0.0475 | 0.0312 | 1.0 | 0.385 |
| 2 | Cypermethrin | y = 7.428832 + 1.650163 X | 4.9073 | 0.0370 | 0.0437 | 0.0261 | 1.19 | 0.370 |
| 3 | Fipronil | y = 7.07279 + 1.090043 X | 2.548 | 0.0125 | 0.0195 | 0.0081 | 3.85 | 0.063 |
| 4 | Imidacloprid | y = 7.156348 + 1.000411 X | 7.2951 | 0.0070 | 0.0106 | 0.0045 | 6.93 | 0.350 |
| 5 | Indoxacarb | y = 7.589628 + 1.644566 X | 3.2034 | 0.0266 | 0.0344 | 0.0258 | 1.20 | 0.443 |

LL – Lower limit; UL – Upper limit

* Heterogeneity: In none of the cases the data were found to be significantly heterogeneous at P=0.05

* y = a+ b log X, where y is probit mortality; X is concentration of insecticides expressed as per cent solutions, a and b are regression coefficients

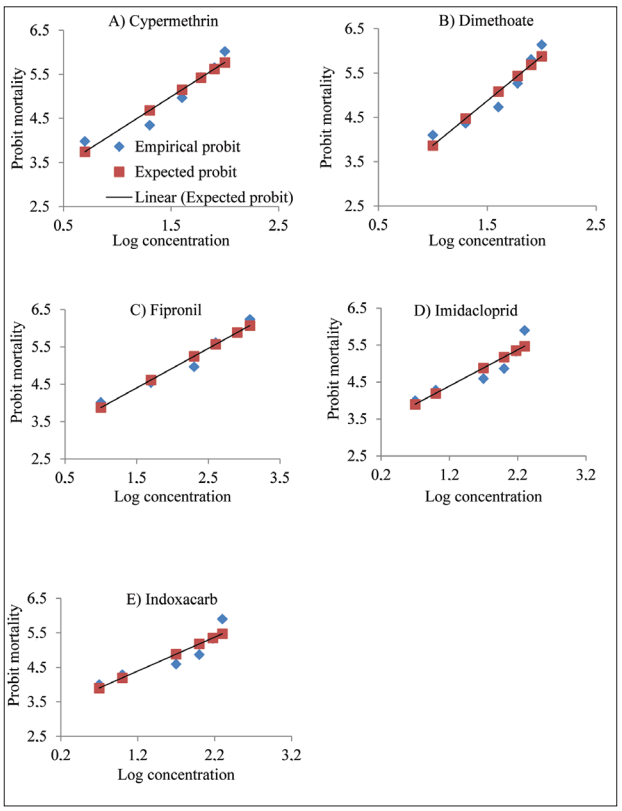


**Fig. 1**. Concentration responsive bioassays were carried out with workers of *A. mellifera mellifera* for cypermethrin (**A**), dimethoate (**B**), fipronil (**C**), imidacloprid (**D**), indoxacarb (**E**). Insecticides were tested at different concentrations and probit mortality was regressed on the log values of insecticide concentration. Calculated regression lines as given in the figure indicate a homogeneous response of honey bee population to insecticides. Regression equations are given in Table 1.

follows: imidacloprid, fipronil, indoxacarb, cypermethrin and dimethoate. The relative toxicity of different insecticides when compared to dimethoate revealed that cypermethrin, indoxacarb, fipronil and imidacloprid were 1.19-, 1.20-, 3.85- and 6.93-foldmore toxic, respectively. The calculated regression lines as given in Table 1 indicate a homogeneous response ($\chi^2$>0.05) of the *A. mellifera mellifera* population to insecticides. The data on regression lines for the tested insecticides are presented in Fig. 1A-E.

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 as follows: 0.063, 0.350, 0.370, 0.385 and 0.443, for fipronil, imidacloprid, cypermethrin, dimethoate and indoxacarb, respectively. According to the safety index, fipronil, followed by imidacloprid, are the least safe to bees.

The obtained $LC_{50}$ values for *A. mellifera mellifera* did not exactly corroborate earlier findings [15,32]. The differences may be due to several factors, such as population origin and age of bees, effect of post treatment temperature, etc. all of which can influence the toxicity of insecticides. However, our findings are in conformity with the highly toxic nature of the tested insecticides observed in the experiment.

The toxic effect of imidacloprid on bees has already been documented [33-35]. The $LC_{50}$ value obtained in *Apis cerana indica* for imidacloprid was 0.0035% [33].Costa et al.[34] determined the topical LD50 for *Melipona scutellaris* was 2.41 ng/bee for 24 h and 1.29 ng/bee for 48 h. The oral LC50 for *M. scutellaris* was 2.01 ng .a. i./µL for 24 h and 0.81 ng a.i./µL for 48 h. Pastagia and Patel [35] obtained 80.67% mortality of the Indian honey bee, *A. cerana*, after application of 0.05% imidacloprid. Imidacloprid was highly toxic to the honeybees as well as wild bees, as reported by Singh [36], Valdovinos-Nunez et al.[37], Scott-Dupree et al. [38] and Lourenco et al. [39]. All these reports lend support to the present finding.

Fipronil was next in the order of toxicity, with an $LC_{50}$ value of 0.0125% in the present investigation. Fipronil is highly toxic to honey bees, as reported by Kim et al. [40]. Jacob et al. [41] also endorsed the toxicity of fipronil to stingless bees (*Scaptotrigona postica* Latreille).

The toxicity of indoxacarb as observed in the present investigation is in agreement with Yu et al. [32] who recorded a $LC_{50}$value of 3.54 mg/L to *A. mellifera*. Cypermethrin was highly toxic to honey bees [42,43], with an$LC_{50}$ value of 0.017270% [44]. Delabie et al.[45] found cypermethrin to be highly toxic to honey bees when topically applied. These earlier findings support the present finding.

The present finding about dimethoate toxicity is in agreement with Abrol and Andotra [46] and Gour and Pareek [44] who observed that dimethoate was highly toxic to bees. Several researchers have reported differences in selectivity among the toxicants. The ob-

**Table 2.** Time required to cause fifty per cent mortality of workers of *A. mellifera mellifera* after treatment of insecticidal formulations (at the recommended dose).

| Sr. No. | Insecticide | Regression equation | $X^{2*}$ | $LT_{50}$ (HAT) | Fiducial limits | |
|---|---|---|---|---|---|---|
| | | | | | LL (HAT) | UL (HAT) |
| 1 | Dimethoate 30% EC | y = 2.806 + 2.428 X | 1.4636 | 8.00 | 6.49 | 9.86 |
| 2 | Cypermethrin 10% EC | y = 3.168 + 2.217 X | 0.8418 | 6.69 | 5.42 | 8.28 |
| 3 | Fipronil 5% SC | y = 2.591 + 2.946 X | 1.5287 | 6.56 | 5.34 | 8.07 |
| 4 | Imidacloprid 17.8% SL | y = 2.344 + 2.674 X | 2.6156 | 9.85 | 7.31 | 13.23 |
| 5 | Indoxacarb 14.5% SC | y = 1.605 + 3.007 X | 3.1123 | 13.45 | 10.86 | 16.66 |

HAT – hours after treatment
LL – Lower limit; UL – Upper limit
*Heterogeneity: * in none of the cases were the data found to be significantly heterogeneous at P=0.05
* y = a+ b log X, where y is probit mortality, X is the time of exposure in h after treatment, a and b are regression coefficients

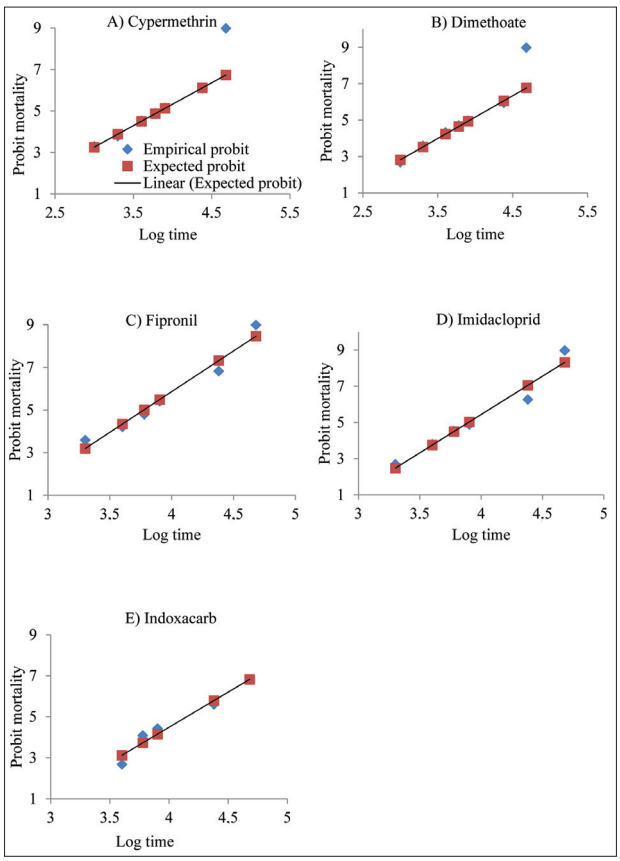


**Fig. 2**. *A. mellifera mellifera* workers were used for the bioassay studies to determine the mortality time for some insecticides. The recommended doses of each formulated insecticide were prepared and applied as a spray to the bees. The mortality of bees was observed at successive intervals of 1, 2, 4, 6, 8, 24 and 48 h after treatment. The data on cumulative mortality (corrected by Abbott's formula) at various time intervals was utilized for calculation of medial lethal time by probit mortality vs. log time. Calculated regression lines as given in the figure indicate a homogeneous response of the honey bee population to insecticides. Regression equations are given in Table 2.

served 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 [26].

## Determination of time mortality (LT50)

The percentage mortality of worker bees was observed after direct spraying with the recommended doses of the respective insecticides. The observations were recorded at successive intervals (up to 48 h after treatment). The corrected mortality observed at different times (h) for the six insecticides and the $LT_{50}$ values are presented in Table 2.

The $LT_{50}$ values in ascending order of toxicity for the different insecticides were as follows: fipronil (6.56 h), cypermethrin (6.69 h), dimethoate (8.00 h), imidacloprid (9.85 h) and indoxacarb (13.45 h). This finding indicates that fipronil was the most toxic, with the lowest $LT_{50}$ value, followed by cypermethrin and dimethoate. The calculated regression equation revealed a homogenous response of *A. mellifera mellifera* to insecticides (Fig. 2A-E).The lower values of $LT_{50}$ pointed to a quick death of the exposed bees due to the highly toxic nature of the insecticides. Azadirachtin was harmless to *A. mellifera mellifera*, which corroborates with earlier studies [47].

The present results are not in complete agreement with the findings of other authors because of the variability of the bee species, different formulations, dif-

**Table 3.** Symptoms observed in worker honey bees (*A. mellifera mellifera*) after direct spraying of insecticides.

| Insecticide/Treatment | Feeding behavior | Symptoms observed up to 8 h after treatment | Symptoms observed in dead bees |
|---|---|---|---|
| Azadirachtin 0.15% | Normal | - | - |
| Dimethoate 30% EC | Normal | Slow movements (later HAT) | Extended proboscis and wing expansion |
| Cypermethrin 10% EC | Normal | Slow movements few minutes before death | Extended proboscis |
| Fipronil 5% SC | Normal | Normal movements (initial HAT), slight trembling and shaking for few minutes just before death | Extended proboscis and full wing expansion, unhooked wings, extended legs, twisted body |
| Imidacloprid 17.8% SL | Normal | Rapid movements (initial HAT) and slow movements (later HAT) | Extended proboscis and full wing expansion, unhooked wings, extended legs |
| Indoxacarb 14.5% SC | Normal | Aggressive rapid movements and stinging and defection (later HAT) | Marked defecation on cage covers, dead bees with sting in release-out position and anus with excreta, extended proboscis and wing expansion, unhooked wings |

HAT – hours after treatment; initial HAT: 2, 4 h; later HAT: 8 h

ferent concentrations and experimental conditions. Husain et al. [48] recorded a toxic effect of imidacloprid (1000 ppm) on *A. mellifera*, with a $LT_{50}$ of 4 h, and indoxacarb (1000 ppm) with a $LT_{50}$ of 6 h. Abrol and Andorta [46] observed 100% mortality in dimethoate-treated bees at 12 h after spraying. All these reports support the present findings.

### Symptoms observed in worker honey bees (*A. mellifera mellifera*) after direct spraying of insecticides

During the bioassay experiment, dead bees in the control acetone-treated sample had wings folded over the body and tongue laid out horizontally beneath the body. Among the tested insecticides, the feeding behavior of *A. mellifera* was normal after exposure but the bees exhibited different symptoms (Table 3; Supplementary Fig. S1). These symptoms are in conformity with earlier reports. The symptoms of bee poisoning, such as stupefaction, paralysis and abnormal rapid movements and spinning on the back are due to toxicity of organophosphates, organochlorines, and neonicotinoids [49-50]. Regurgitation of stomach contents and tongue extension are attributed to organophosphates and pyrethroids [2, 51]. Suhail et al. [52] observed rapid neurotoxic symptoms, such as coordination problems, trembling and tumbling after exposure with imidacloprid. The extended tongue, detached fore and hind wings and asymmetric legs in honey bees were observed earlier due to acephate toxicity [53].

## CONCLUSION

All the tested pesticides were harmful to the honey bee (*A. mellifera mellifera*), except azadirachtin. The pesticides exhibited different poisoning symptoms in bees, which could be useful for beekeepers for the identification of the cause of colony mortality. Efforts in health risk assessment studies may be regarded as an aid towards a better understanding of the problem. Our data revealed a range of $LC_{50}$ and $LT_{50}$ values for used formulated pesticides, suggesting that the chemical risk to bee pollinators could be minimized by the choice of pesticides with lower toxicity for bees in crop pest management.

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**Conflict of interest disclosure:** The authors declare that there are no conflicts of interest.

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**Supplementary Fig. S1**. Symptoms of bee poisoning after exposure to different insecticides.
The results are available via the link:
http://serbiosoc.org.rs/sup/4/Pashte_and_Patil_1441_FigS1.pdf