ACUTE TOXICITY OF SOME INSECTICIDES ON HONEYBEE, Apis mellifera L.

Mona H.I. Radwan1*, R.E. Sand2 and M.A. Hendawy3

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ABSTRACT: This work was conducted to evaluate the toxicity of some insecticides against honeybee workers under laboratory conditions. Among the tested insecticides, Deltamethrin was the most toxic compound followed by Methomyl while Chlorpyrifos was the least one followed by Profenfos after 24, 48, and 72 hr., of topical exposure. The results indicated that there was negative relationship between the time post treatment and lethal concentration (LC50) values of all the tested insecticides. The LD50 values were 9.5, 10.45, 3.68, 18.33 and 12.55 µg/bee for Methomyl, Cyhalothrin, Deltamethrin, Chlorpyrifos and Profenfos, respectively after 24 hr., of exposure. In this respect, the toxicity of the insecticide Deltamethrin was 2.58 times more than Methomyl, 2.83 times than Cyhalothrin, 4.98 times than Chlorpyrifos and 3.41 times than Profenfos. On the other hand, the toxicity of Methomyl was 1.93 times, more than Chlorpyrifos 1.37 times than Profenfos and 1.1 times than Cyhalothrin. The toxicity of the tested insecticides against the workers of honeybee after 48 hr., of topical exposure showed LD50 values range from 2.44 to 12.32 µg/bee. The insecticide Deltamethrin was the highest toxic compound where as the Chlorpyrifos was the least toxic one. The other insecticides occupied intermediate degree of toxicity. Deltamethrin toxicity in comparision to the other tested insecticides revealed that it was more toxic than Methomyl by 2.21 times, 2.93 times than Cyhalothrin, 5.01 times than Chlorpyrifos and 3.82 times than Profenfos. The LD50 of Deltamethrin was 1.01 mg/l, 0.1mg/l to Methomyl, 5.21 µg/bee to Cyhalothrin, 7.58 mg/l to Chlorpyrifos and 6.11 mg/l to Profenfos. On the other hand, the corresponding LD90 were 2.31 to Deltamethrin, 8.71 to Methomyl, 9.98 to Cyhalothrin, 13.75 to Chlorpyrifos and 15.73 µg/bee to Profenfos. The tested insecticides could be arranged descendingly as follows: Deltamethrin > Methomyl > Cyhalothrin > Profenfos > Chlorpyrifos.

Key words: Insecticides, toxicity, honeybee, Apis mellifera.

INTRODUCTION

Pesticides are used to protect agricultural crops, however, some may affect bee health, with their toxicity depending on the active substance used and the formulation of different pesticide products. The risk of influencing bee health also increases when, amongst other potential factors, legal requirements or good practice for pesticide application have not been followed. Bees can be exposed to pesticides when spray drifts onto non target fields of crops in bloom, or nearby wildflowers or beehives, contaminating pollen and nectar. In cases where exposure is suspected, it is necessary to analyse dead bee samples and identify the cause of death and the pesticides possibly involved (Kozowicka, 2013).

Insecticides are important for ensuring both crop quality and quantity in today’s integrated crop management for sustainable agricultural production. The use of insecticides is one of the most effective practices to control pests.
However, what concerning us most is how residual levels of sub-lethal dosages of those insecticides being used lead to detrimental effects on non-target pollination species of honeybee and its development, foraging behavior and colony conditions. Either wild or domesticated honeybee, *Apis mellifera*, has been recognized and used as a major pollinator in the agricultural system (Kevan, 1999) and by beekeepers to produce valuable products such as honey, royal jelly and pollen. However, honeybee rely on flower plants while foraging and collecting its food sources of nectar and pollen and thus at risk endangering exposing to various levels of chemical residues of pesticides while they are collect nectar and pollen (Peach et al., 1993).

Honeybee workers may be poisoned by the residual pesticides on the nectar and pollen they collect. In addition, the workers may take the pesticide-contaminated nectar and pollen back to their hive. This will expose the larvae, drones and queen to these pesticides, and eventually poison them and causes high mortality.

Feeding honeybee larvae on contaminated nectar and pollen transmitted from the sprayed fields to the hive may also be considered another destructive agents to the honeybee colony, there is scarce data about insecticides toxicity in honey bee brood of neem oil and some insect growth regulators as well as a few number of canceled pesticides (Erickson, 2013; Pashte and Patil, 2017).

From this stand point the present work was designed to assess the topical, as well as the initial and residual activity of some pesticides against honeybee workers.

**MATERIALS AND METHODS**

**Chemicals Used**

**Carbamate compound**

Methomyl [Lannate 90% WP]. Ethanimidothioic acid, N-[methylamino carponyloxy] thio methyl.

The recommended field rate is 300 grams/faddan.

**Organophosphorus compounds**


The recommended field rate is 1 liter/faddan.

B- Profenofos [Selecron EC 72%]. O-[4-bromo-2-chlorophenyl] O-ethyl –S-propyl phosphorothioate.

The recommended field rate is 750 ml/faddan.

**Synthetic Pyrethroid Compounds**

A- Cyhalothrin (Lambda EC 5%). cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropene-carboxylate.

The recommended field rate is 750 ml/faddan.

B- Deltamethrin (Decis EC 2.5%). (S)-a-cyano-m-Phenoxybenzyl (1R 3R)-3-(2,2 dimethyl cyclopeopane carboxylate).

The recommended field rate is 750 ml/faddan.

**Rearing of Insect**

The 2nd of honeybee workers needed for laboratory tests were collected from the peripheral combs of the colony. To minimize the genetic variations as possible, tested workers were collected from the honey chamber (hive) of one colony headed by open mated F1 Carniolan queen from the educational Apiary of Faculty of Agriculture, Zagazig University.

**Laboratory Experiments**

**Acute toxicity of the tested insecticides**

**Topical application technique**

The toxicity of the tested insecticides against honeybee workers was evaluated using the topical application method (Stevenson, 1968). Bees of approximately identical weight and age were slightly anesithized by chilling (5 minutes in deep freezer). One µl acetone solution of insecticidal dilution was administered individually on the thoracic mesonotum using the syringe of micro applicator. The control workers were treated with acetone only. The doses used in this study were 1, 10 and 30 µg/bee for Methomyl and Cyhalothrin, 0.1, 1 and 10 µg/bee for Deltamethrin and 1, 20 and 50 µg/bee for Chlorpyrifos and Profenofos.

At least three concentrations and 30 workers were used for each insecticide after application, bees of each concentration were placed together in small feeding cages of 10×10×15 cm and fed
on 1:1 sugar syrup. The tests were carried out during late summer (2017) at room temperature (25 – 27°C), and RH (60-68%).

The corrected mortality of bees was carried out using Abbott's formula (Abbott, 1925). The LC$_{50}$, LC$_{90}$ and slope values of the tested compounds were calculated using Finney equation (Finney, 1971) through software computer program.

Toxicity index and relative potency calculated according to Sun equations (Sun, 1950) as following:

Toxicity index =

\[
\frac{LC_{50} \text{ or } LC_{90} \text{ of the most efficient compound}}{LC_{50} \text{ or } LC_{90} \text{ of the tested compound}} \times 100
\]

Relative potency =

\[
\frac{LC_{50} \text{ or } LC_{90} \text{ of the tested compound}}{LC_{50} \text{ or } LC_{90} \text{ of the most efficient compound}} = \text{fold}
\]

RESULTS AND DISCUSSION

Efficacy of the Tested Insecticides Against Honeybee Workers Under Laboratory Conditions

After 24 hour.

The results presented in Table 1 reveal that the toxicity of five insecticides; i.e. Methomyl, Cyhalothrin, Deltamethrin, Chlorpyrifos and Profenfos against the 2nd honeybee workers, *Apis mellifera* L for 24 hr., exposure time. Among the tested insecticides Deltamethrin was the most toxic compound followed by methomyl while Chlorpyrifos was the least one followed by Profenfos.

The results indicated that there was negative relationship between the time post treatment and LD$_{50}$ values of all the tested insecticides. The LD$_{50}$ values were 0.95, 1.045, 0.36, 1.83 and 1.25 µg/bee for Methomyl, Cyhalothrin, Deltamethrin, Chlorpyrifos and Profenfos after 24 hr., of exposure.

In this respect, the toxicity of the insecticide Deltamethrin was more than Methomyl by 2.58 times, 2.83 times than Cyhalothrin, 4.98 times than Chlorpyrifos and 3.41 times than Profenfos. On the other hand, the toxicity of Methomyl was more than Chlorpyrifos by 1.93 times, 1.37 times than Profenfos and 1.1 times than Cyhalothrin.

After 48 hour

The toxicity of the tested insecticides against the workers of honeybee after 48 hr., were recorded in Table 2. The LD$_{50}$ values ranged between 0.24 and 1.23 µg/bee. The insecticide Deltamethrin was the highest toxic compound while the Chlorpyrifos was the lowest toxic one. Other insecticides occupied intermediate toxicity. Results showed that toxicity of Deltamethrin was more than Methomyl by 2.21 times, 2.93 times than cyhalothrin, 5.01 times than chlorpyrifos and 3.82 times than Profenfos.

After 72 hour

Results presented in Table 3 show that the LD$_{50}$ of Deltamethrin was 0.1 µg/bee, 0.62 µg/bee to Methomyl, 0.51 µg/bee to Cyhalothrin, 0.75 µg/l to Chlorpyrifos and 0.61 µg/l to Profenfos. The corresponding LD$_{90}$ were 0.23 to Deltamethrin, 0.81 to Methomyl, 0.99 to Cyhalothrin, 1.37 to Chlorpyrifos and 1.57 to Profenfos.

The tested insecticides could be arranged descendingly as follows: Deltamethrin > Methomyl > Cyhalothrin > Profenfos > Chlorpyrifos.

In connection to our finding, Benedek (1983) found that the direct toxicity to honeybee against 5 synthetic pyrethroid insecticides, penthrin, chinthrin, cypermethrin, fenvalerate and dethylmethrin was high. Kasamatsu and Kawachi (1985), Erickson (2013) and Pashte and Patil (2017) reported that the LD$_{50}$ value of dethylmethrin recorded 0.004 µg/bee.

Wilkinson et al. (1986) reported that cyhalothrin (Kur.) is toxic to honeybees in laboratory tests. Mayer et al. (1990) found that the LD$_{50}$ value of fenvalerate for honeybees was 0.471 µg/g body weight and EldAnsary and EldZogby (1992) stated that the LD$_{50}$ of dethylmethrin (Decis) was 0.014 µg /bee being greatly higher than that recorded in the present work. This variation may be due to the varied sensitivity of the varied races treated. Also, lab. conditions surely are different. In addition they did not give any information about the formulation they tested.
Table 1. Acute toxicity of the tested insecticides on honeybee workers 24 hr., after treatment

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>LD(_{50}) ug/bee</th>
<th>LD(_{90}) ug/bee</th>
<th>Slope</th>
<th>Toxicity index</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>0.95</td>
<td>1.63</td>
<td>1.66</td>
<td>38.47</td>
<td>2.58</td>
</tr>
<tr>
<td>Cyhalothrin</td>
<td>1.045</td>
<td>2.21</td>
<td>1.01</td>
<td>35.22</td>
<td>2.84</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>6.36</td>
<td>7.8</td>
<td>1.53</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>1.83</td>
<td>2.8</td>
<td>0.89</td>
<td>20.08</td>
<td>4.98</td>
</tr>
<tr>
<td>Profenfos</td>
<td>1.25</td>
<td>3.3</td>
<td>1.22</td>
<td>29.32</td>
<td>3.41</td>
</tr>
</tbody>
</table>

Table 2. Acute toxicity of the tested insecticides on honeybee workers 48 hr., after treatment

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>LD(_{50}) ug/bee</th>
<th>LD(_{90}) ug/bee</th>
<th>Slope</th>
<th>Toxicity index</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>0.54</td>
<td>1.221</td>
<td>1.06</td>
<td>45.19</td>
<td>2.21</td>
</tr>
<tr>
<td>Cyhalothrin</td>
<td>0.71</td>
<td>1.51</td>
<td>1.55</td>
<td>34.17</td>
<td>2.93</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.24</td>
<td>0.46</td>
<td>1.88</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>1.23</td>
<td>1.73</td>
<td>2.11</td>
<td>19.81</td>
<td>5.01</td>
</tr>
<tr>
<td>Profenfos</td>
<td>0.93</td>
<td>0.71</td>
<td>1.78</td>
<td>26.18</td>
<td>3.82</td>
</tr>
</tbody>
</table>

Table 3. Acute toxicity of the tested insecticides to honeybee workers 72 hr., after treatment

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>LD(_{50}) ug/bee</th>
<th>LD(_{90}) ug/bee</th>
<th>Slope</th>
<th>Toxicity index</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>0.62</td>
<td>0.81</td>
<td>1.33</td>
<td>30.79</td>
<td>3.25</td>
</tr>
<tr>
<td>Cyhalothrin</td>
<td>0.51</td>
<td>0.99</td>
<td>0.98</td>
<td>19.39</td>
<td>5.16</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.1</td>
<td>0.23</td>
<td>1.38</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.75</td>
<td>1.37</td>
<td>1.21</td>
<td>13.32</td>
<td>7.50</td>
</tr>
<tr>
<td>Profenfos</td>
<td>0.61</td>
<td>1.57</td>
<td>1.55</td>
<td>16.53</td>
<td>6.05</td>
</tr>
</tbody>
</table>

In this respect, Matar (1996) recorded LD\(_{50}\) and LD\(_{90}\) values (µg/bee) of fenvalerate (0.0030 and 0.029). Ebadah (1998) recorded LD\(_{50}\) and LD\(_{90}\) values (µg/bee) as following cyhalothrin (Karate) (0.026 and 0.082), cyhalothrin (Karate super) (0.0207 and 0.0858) and fenpropathrin (0.0345 and 0.1844), respectively.

Also, Wael and Van (1989) reported that fenvalerate (Sumicidin), alpha cypermethrin (Pastac) and tenpropathrin (Danitol) were highly toxic to honeybee in feeding. Moreover, Ebadah (1998) found that the LC\(_{50}\) for cyhalothrin (Karate) and tenpropathrin by ingestion on honeybee workers were 25.68 and 37.38 ppm., respectively. Similarly, Gromisz and Cromisz (1996) reported that Bulldock (beta-cyfluthrin) is highly toxic to bees when ingested the lethal dose of the active ingredient a.i. 0.2-0.3 ppm/bee.

The severe toxicity of synthetic pyrethroids by ingestion to honeybee was reported by many
authors (Arzone and Patetta, 1982; Arzone and Vidano, 1985; Atallah et al., 1989; Matar, 1996; Ebadah, 1998).

It is obvious that within the same group there are highly toxic and less toxic compounds. Moreover, the two formulations (Karate and Kendo) of the same compound (Cyhalothrin) showed varied oral toxicity to honeybee workers. The differed toxicity of the formulations was reported by Atallah et al. (1989). Their variations could be attributed to the synergistic, dispersing and solved materials added to the formulations which increased or decreased toxicity.

Results of the present work are in agreement with those of Arzone and Patetta (1982) who reported that, fenpropatarin was highly toxic stomach poisons to honeybee. Also, Arzone and Patetta (1987, 1991) reported that flucythrinate was moderately toxic on ingestion and cyfluthrin was markedly toxic in feeding to honeybee.

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