INTRODUCTION

The neonicotinoids currently on the market can be divided into two subclasses: Chloronicotinyl, which includes Acetamiprid, Imidacloprid and Thiacloprid, and Thianicotinyl with Clothianidin and Thiametoxam. All active ingredients show a systemic action and their action is directed towards a wide range of insects on vegetable crops, fruit, flowers, and others. More specifically, Imidacloprid, Clothianidin, and Thiametoxam for their excellent systemic proprieties are also widely used in seed dressing. Such active ingredients spread into the surrounding soil, thus creating a protective barrier that can control several soil insects and prevent the damage that they can cause (Muccinelli, 2008; Maini et al., 2010). Once they are absorbed by the roots, they translocate in the young plant maintaining a concentration of the active ingredient that protects the plant for a long period of time.

Neonicotinoids are active both by direct contact and ingestion against many insect pests with chewing, piercing, and sucking mouthparts, by blocking acetylcholine receptors in the post-synaptic button, thereby stopping the nerve impulse and resulting in the insect’s death (Tomizawa and Casida, 2005; Maccagnani et al., 2008; Muccinelli, 2008).

Toxicity occurs also against useful insects, especially the honey bees, with a mortality that is not always easily detectable because it can even occur in the open field during their foraging activity.

It was therefore considered appropriate to undertake experimental studies to assess, through the calculation of LD₅₀, not only the danger of the three active ingredients which are most used in seed dressing for honey bees, but also to highlight possible differences in sensitivity between three strains of Apis mellifera ligustica Spinola bees of the Piedmontese origin.

MATERIAL AND METHODS

Commercial formulations available in Italy were used. They contained: Clothianidin (Dantop 50 WG: 50.0% pure a.i., hydro dispersible granules), Imidacloprid (Confidor 200 SL: 17.8% pure a.i., concentrated liquid soluble in water), Thiametoxam (Actara 25 WG: 25.0% pure a.i., hydro dispersible granules).

Experiments were carried out using the methods designed to test acute oral toxicity of insecticides towards the honey bee (Argone and Vidano, 1980). Compounds were tested at the highest concentration recommended on the label for crop treatment (field concentration) and they were gradually diluted down to the concentration that caused mortality not significantly different from that of the untreated controls in the case of mortality higher than that of the untreated controls.

Laboratory tests were conducted using cages of 20x20x30 cm with the bottom and two opposing walls in
The honey bees were administered a 25% sucrose solution, pure for untreated controls and in the other cases known amounts of the compounds to be tested were added. Solutions were administered through a feeder made of a 7 mm high and 28.2 mm internal diameter capsule in which an inverted 25.9 mm wide and 70 mm long test tube was placed. The resulting 1.15 mm annular space allowed the workers to suck the liquid, but prevented contact with the legs. Solutions were made available to the honey bees for one hour and the feeders were later covered with plastic glasses. The honey bees could feed only on sugar candy throughout the remaining part of the trial (ARZONE and VIDANO, 1974).

Tests started at 12.00 h; mortality was checked at 13.00 h, 15.00 h, and 18.00 h on the first day of the trial and at 9.00 h, 12.00 h, 15.00 h, and 18.00 h during the following days.

Tested a.i. concentrations were:
- Clothianidin: 75 ppm, 0.75 ppm, 0.375 ppm, 0.15 ppm, 0.075 ppm, 0.0375 ppm, 0.015 ppm, 0.0075 ppm;
- Imidacloprid: 150 ppm, 15 ppm, 7.5 ppm, 3 ppm, 1.5 ppm, 0.75 ppm, 0.3 ppm, 0.15 ppm;
- Thiametoxam: 100 ppm, 1 ppm, 0.5 ppm, 0.2 ppm, 0.1 ppm, 0.05 ppm, 0.02 ppm, 0.01 ppm.

STATISTICAL ANALYSIS
Forty honey bees were used for each a.i. at each concentration and for the untreated controls. The number of dead and living honey bees was compared with that of the relative control group with the chi-square test. Only the counts done after 1, 3, 6, 24, 48, and 72 hours from the beginning of the trials were statistically checked.

Acute oral LC<sub>50</sub> were calculated by means of probit analysis. Considering that each honey bee ingested 35 µl of sucrose syrup during the allowed one hour feeding period, the acute oral LD<sub>50</sub> was obtained from the relative LC<sub>50</sub>.

RESULTS

During the trials, the honey bees showed obvious symptoms of poisoning, such as shaking and tremors, uncoordinated and uncontrolled movements, inability to take up a correct position of the body, and prolonged frenetic movement of the legs and rotation when being in the supine position. Direct observation of the behaviour of the honey bees in cages proved that it was transient at a lower concentration. Moreover, the highest concentrations caused extensive vomiting by honey bees.

Mortality was similar in all three strains of treated bees. Clothianidin caused the death of all the tested honey bees within 3 h from the start of the trial at the field concentration of 75 ppm, and was still toxic within 72 h at the concentration of 0.015 ppm, 5000 times lower. Mortality at the concentration of 0.75 ppm 1 h after the beginning of the test was similar to that at the concentration of 75 ppm. This phenomenon was consistent for all three colonies (fig. II).

The graph shows that Imidacloprid at the field dose kills 100% of the tested bees 6 hours after the start of the experiment. At doses 20 or more times lower than the field dose, the three strains showed different responses and mortality decrease was not always proportional to the administered doses (fig. III).

Thiametoxam caused the death of all the tested honey bees even at the concentration of 0.5 ppm, 200 times less than the field concentration, within 6 h after the beginning of the test. This product caused a statistically significant mortality up to 0.05 ppm in two strains and up to 0.02 ppm in the third; such a concentration is 5000 times lower than the field one (fig. IV).

Acute oral LD<sub>50</sub> was calculated for Clothianidin, Imidacloprid and Thiametoxam at 24, 48, and 72 hours after the beginning of the test (Table 1).
DISCUSSION

Poisoning symptoms similar to those observed in the trials had already been reported for various neonicotinoids (BORTOLOTTI et al., 2003; MEDRZICKY et al., 2003; MACCAGNANI et al., 2008; SUCHAIL et al., 2001). The highlighted disabling behaviour, although transient for some active ingredients at low concentrations, could irreversibly affect honey bee survival in the field taking

Table 1 – LD₅₀ values (ng/bee) at the different times for the three active ingredients.

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Beehive 1</th>
<th>Beehive 2</th>
<th>Beehive 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clothianidin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>4.930</td>
<td>3.885</td>
<td>4.627</td>
</tr>
<tr>
<td>48 h</td>
<td>4.671</td>
<td>3.789</td>
<td>4.507</td>
</tr>
<tr>
<td>72 h</td>
<td>4.514</td>
<td>3.747</td>
<td>4.369</td>
</tr>
<tr>
<td><strong>Imidacloprid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>191.044</td>
<td>173.088</td>
<td>187.208</td>
</tr>
<tr>
<td>48 h</td>
<td>99.063</td>
<td>103.705</td>
<td>109.579</td>
</tr>
<tr>
<td>72 h</td>
<td>74.631</td>
<td>46.763</td>
<td>97.425</td>
</tr>
<tr>
<td><strong>Thiametoxam</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>2.761</td>
<td>3.336</td>
<td>4.546</td>
</tr>
<tr>
<td>48 h</td>
<td>2.644</td>
<td>3.018</td>
<td>4.383</td>
</tr>
<tr>
<td>72 h</td>
<td>2.556</td>
<td>2.936</td>
<td>3.151</td>
</tr>
</tbody>
</table>

Fig. II – Clothianidin effects on honey bees.

Fig. III – Imidacloprid effects on honey bees.

Fig. IV – Thiametoxam effects on honey bees.
into account external dangers that may occur, such as cold and predation. Moreover, even if the poisoned honey bees managed to return to the colony, their memory and communication abilities might be impaired (Desneux et al., 2007; Maccagnani et al., 2008).

The graphs are somehow irregular (in particular with Imidacloprid) with some lines overlapping, conceivably due to the observed vomiting reaction. This very likely reduced a.i. absorption by the honey bees, thus slightly extending their life span while not guaranteeing their survival.

Test results presented in this paper are in line with those reported in the literature (Bailey et al., 2005; Muccinelli, 2008).

Clothianidin and Thiametoxam are highly toxic even if the latter is somehow less dangerous at reduced concentrations. The calculated LD$_{50}$ acute oral toxicities are in accordance with those reported in the literature (Tomlin, 1994). LD$_{50}$ data for Imidacloprid instead quite variable (C. Doucet-Personeni et al., 2003). Our results are comparable with the highest values reported by Suchail et al. (2001). There is a repellent effect also reported for Imidacloprid (Ramirez-Romero et al., 2005). If this were to be the case, and without taking any sublethal effects into account, some hazards could arise when colonies are severely short of stores or after prolonged seclusion.

ACKNOWLEDGEMENTS

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RIASSUNTO

TOSSICITÀ ACUTA ORALE DI NEONICOTINOIDI SU DIFFERENTI CEPPI DI APE

I neonicotinoïdici costituiscono una classe di insetticidi di concezione relativamente nuova, studiata a partire dagli anni ’80, per le interessanti prospettive che potevano avere in quanto altamente sistemicì e di lunga persistenza. Questi insetticidi manifestano però una tossicità molto spiccata per gli insetti prouni e per l’ape, provocando anche altri effetti, spesso di non facile individuazione, quali turbine comportamentali, difficoltà di orientamento e alterazione delle attività sociali.

Negli ultimi anni, in molti Paesi, sono stati registrati allarmanti fenomeni di mortalità di api, chiaramente riconducibili in alcuni casi, all’impiego di neonicotinoïdici sia come concianti sia come prodotti fitosanitari.

Si è pertanto ritenuto opportuno verificare in laboratorio la tossicità acuta orale dell’Imidacloprid, in commercio da anni, e di Clothianidin e Thiametoxam di più recente introduzione in Italia, nei confronti di tre ceppi di Apis mellifera di provenienza Piemontese e riferibili ad A. m. ligustica.


Quando nelle api in prova veniva osservata una mortalità superiore a quella manifestata dai testimoni non trattati, venivano saggiate concentrazioni via via decrescenti sino al raggiungimento di una mortalità non significativamente diversa da quella riscontrata nei testimoni.

Le DL$_{50}$ calcolate per Clothianidin e Thiametoxam sono risultate nettamente inferiori a quelle dell’imidacloprid; le differenze riscontrate tra i diversi ceppi di ape per il medesimo principio attivo appaiono invece moderate.

REFERENCES


