ACUTE ORAL TOXICITY OF NEONICOTINOIDS ON DIFFERENT HONEY BEE STRAINS

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ACUTE ORAL TOXICITY OF NEONICOTINOIDS ON DIFFERENT HONEY BEE STRAINS ([1])

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Laurino D., Manino A., Patetta A., Ansaldi M., Porporato M. – Acute oral toxicity of neonicotinoids on different bee strains.

Neonicotinoids are a class of relatively new insecticides, designed in the '80s, characterized by their excellent feedback, since they are highly systemic and with long-term persistence. These insecticides, however, show very strong toxicity to pollinating insects and in particular to the honey bee, besides causing also other effects which are often not easily identifiable, such as behavioural disturbances, orientation difficulties, and impairment of social activities.

During the past few years, in many countries, alarming bee mortality rates were recorded. In some cases this was clearly due to the use of neonicotinoids either for seed dressing or crop spraying. It was therefore considered appropriate to test on three strains of *Apis mellifera* of Piedmontese origin and referable to *A. m. ligustica* in the laboratory both the acute oral toxicity of Imidacloprid, which had been, in use for many years, and Clothianidin and Thiametoxam, only more recently introduced in Italy.

To reach this aim, methods developed at the Di.Va.P.R.A. and applied in several previous studies, were used. Commercial products were used throughout the tests. They were tested at the recommended concentration for field treatments. When mortality was higher in the tested bees than in the untreated ones, the active ingredients were tested at gradually decreasing concentrations so as to reach a mortality not significantly different from that found in the untreated controls.

The LD_{50} calculated for Clothianidin and Thiametoxam were lower than for Imidacloprid; the differences between the honey bee strains for the same active ingredient were slight.

KEY WORDS: poisoning, Clothianidin, Imidacloprid, Thiametoxam.

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 LD_{50}, 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 (ARZONE 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

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transparent Plexiglas and the other two walls made of a nylon net mesh of 300 µm. Once equipped with the necessary materials for the tests, the cages were closed with a nylon net lid, which was never removed during the test. Honey bee release and all other activities were performed through a circular opening in a wall net, with a 10 cm diameter, connected to a 20 cm long sleeve.

For each tested bee strain, ten foraging bees taken from the flight board were placed in each cage, not later than 15 minutes from capture (fig. I). The hives they came from had been periodically checked to exclude the presence of the most common honey bee diseases. Tests were performed in a dark room at 28-30 °C and 70% relative humidity.

Fig. I – Cage prepared for the ingestion trials (left); detail of the feeder pointing out the narrow space where the bees can feed (centre); after 1 hour the feeder is covered and the bees can feed on some candy (right).

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_{50} 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_{50} was obtained from the relative LC_{50}.

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_{50} was calculated for Clothianidin, Imidacloprid and Thiametoxam at 24, 48, and 72 hours after the beginning of the test (Table 1).

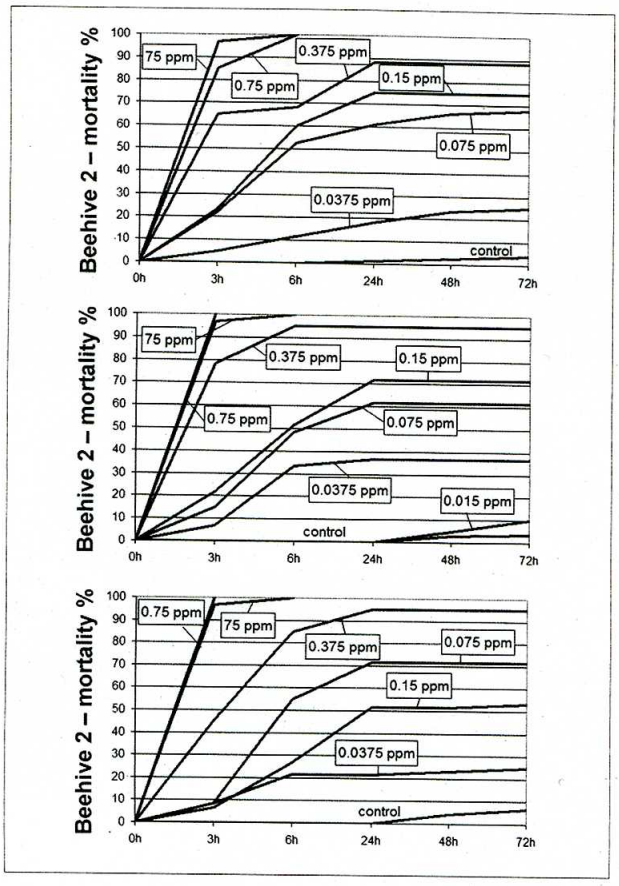

Fig. II – Clothianidin effects on honey bees.

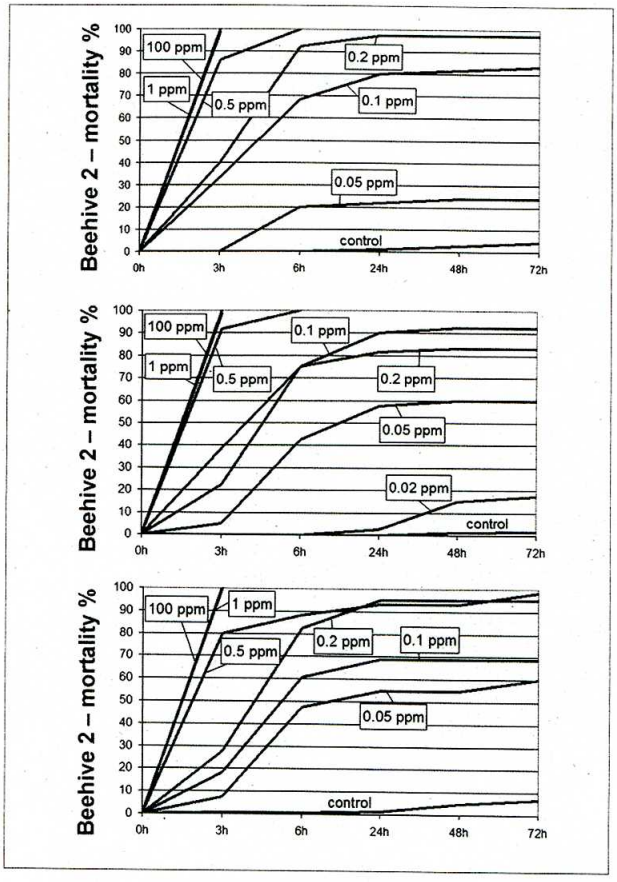

Fig. IV – Thiametoxam effects on honey bees.

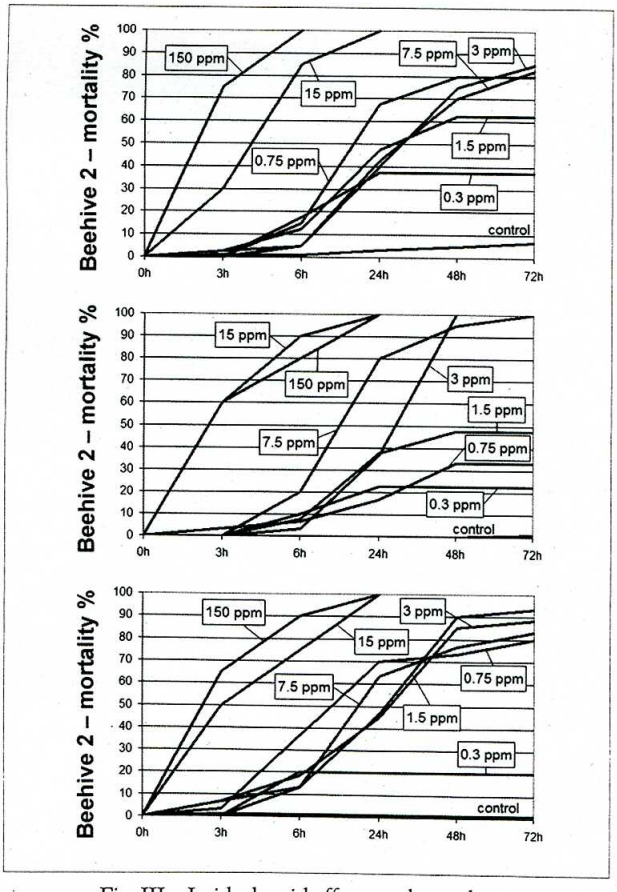

Fig. III – Imidacloprid effects on honey bees.

Table 1 – LD_{50} values (ng/bee) at the different times for the three active ingredients.

	Beehive 1	Beehive 2	Beehive 3
	Clothianidin		
24 h	4.930	3.885	4.627
48 h	4.671	3.789	4.507
72 h	4.514	3.747	4.369
	Imidacloprid		
24 h	191.044	173.088	187.208
48 h	99.063	103.705	109.579
72 h	74.631	46.763	97.425
	Thiametoxam		
24 h	2.761	3.336	4.546
48 h	2.644	3.018	4.383
72 h	2.556	2.936	3.151

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

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 neonicotinoidi 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 sistemici e di lunga persistenza.

Questi insetticidi manifestano però una tossicità molto spiccata per gli insetti pronubi e per l'ape, provocando anche altri effetti, spesso di non facile individuazione, quali turbe 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 neonicotinoidi 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*.

A tal fine sono state utilizzate le metodiche messe a punto presso il Di.Va.P.R.A., già applicate in numerosi lavori precedenti, impiegando formulati commerciali. Gli stessi sono stati saggiati alla concentrazione consigliata per i trattamenti in campo.

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 modeste.

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